EXPERIENTIA

Volume 41/No. 5 Pages 559–700 15 May 1985

Reviews

Half a century of stress research: a tribute to Hans Selye by his students and associates*

Hans Selye died in 1982 in Montreal, Canada, at the age of 75. A native of Austria and Hungary, he did a fellowship at Johns Hopkins University, then went to McGill University for more than a decade of research and subsequently he founded the Institute of Experimental Medicine and Surgery at the University of Montreal in 1945. He remained at the Institute until his forced retirement one year prior to his death. Towards the end of his career he established the International Institute of Stress where he was active virtually until the last few weeks of his life.

By his own account, his ideas and early experiments substantially preceded the historical short report on 'A syndrome produced by diverse nocuous agents' published in the 4 July 1936 issue of 'Nature'. Hence, it is appropriate to label his efforts as a half a century quest for the characterization and elucidation of biologic stress.

On the first anniversary of Dr Selye's death his former students and coworkers gathered in Montreal to commemorate the scientific achievements that can be associated with Dr Selye's institute. The program of this two-day Symposium Hans Selye consisted of historical interludes and brief reviews on ongoing research by those who are still active in investigative work. Two special lectures were presented by Dr Julius Axelrod of the National Institutes of Health and Dr Judah Folkman of Harvard Medical School. Both of these prominent scientists knew Dr Selye quite well personally and they contributed significantly to the success of the symposium. The organizing committee of the symposium consisted of Drs André Robert, Sandor Szabo and Yvette Taché.

The present tribute consists of a few historical reviews by graduates of Dr Selye's institute and a series of brief scientific overviews by his former students and associates. The breadth and diversity of these presentations also illustrate the wide scientific basis of research related to biologic stress as well as the flexibility guaranteed by training in Dr Selye's famous institute.

Contents

* Reprints of the review in its entirety are available from Dr. S. Szabo, Department of Pathology, Brigham and Women's Hospital, 75 Francis Street, Boston (MA 02115, USA)

A personal reminiscence of Hans Selye*

by R. Guillemin

Laboratories for Neuroendocrinology, Salk Institute, La Jolla (CA 92138-9216, USA)

I first met Hans Selye in the spring of 1948 when he came to Paris to lecture, in French, at the old hospital of La Pitié. Since the early 1920s La Pitié had been the seat of the annual symposium covering, over the years, the hottest topics of medicine at that particular time. I think it was the first year when the symposium resumed, having been interrupted per force during the somber years of the Second World War. Selye gave several lectures to a packed auditorium on his general adaptation syndrome

*Reproduced with permission from Laboratory Investigation *48* (1984) 367–368.

and his concept of the diseases of adaptation. The magnetism of the man was extraordinary. Selye was then in his mid-forties, a superb lecturer, obviously at ease with the subject that he knew well, since he had created it de toutes pièces, and about which he spoke with passion. Selye was then at the peak of his reputation and influence in modern medicine. The concept that stress, or rather an overreaction by the pituitary-adrenal system to the stress of modern life and environment, could be considered as the cause of many of the diseases for which we had no specific causative agent, such as rheumatoid arthritis, hypertension, myocardial infarctions, and strokes, as presented by Selye was terribly appealing. What was really exciting was that Selye presented experimental replicates of the clinical diseases and lesions by injecting laboratory animals with large doses of the adrenal steroid, desoxycorticosterone, or crude extracts of lyophilized pituitary gland. If, on top of that, the animals had one kidney removed surgically and were given 1% NaCl as drinking fluid then, after a few weeks administration of the steroid or of the crude pituitary extracts, the rats would become hypertensive and show 'Aschoff's nodules' in the myocardium, typical lesions of periarteritis nodosa in the vessels of mesentery, brain hemorrhages, etc. Moreover, the same type of lesions could be seen if the animals were exposed chronically to a continuous stress. Acute stress would stimulate pituitary-adrenal functions and produce gastric ulcers (the alarm reaction), to which the animals would adapt with healing of the acute lesions (period of adaptation), finally to become ill again and succumb with the recurrence of one or another of the lesions mentioned (stage of exhaustion).

All of this was presented with elegance and with a profusion of beautifully illustrated slides of diagrams or histologic preparations. As a young physician just out of medical school, whose medical education had been entirely directed to what was the practice of medicine during those bleak years of medical schooling in Nazi-occupied France, I had the audacity to talk to Selye at the end of one of his lectures and ask him whether I could somehow go to his laboratory at the University of Montréal, where he had just moved from McGill, and study with him for a year or two. Later that year I was in Selye's laboratory, the Institute for Experimental Medicine and Surgery, with a modest fellowship based on some of Selye's own research funds. Life in Selye's laboratory with the 20 or so younger people from all over the world who were studying under him was just as exciting as had been those lectures by him in Paris. The young assistants, as well as the many visitors would follow Selye on the daily rounds by the animal cages where he would observe animals in experimentation, show an incipient arthritis, estimate the degree of renal hypertrophy by gentle palpation, and eventually end up in the 'autopsy room'. There, the technicians would have prepared on the horseshoe table 80 rats, in 10 groups of eight, which had received various treatments. Selye, in the center of the horseshoe table (and of everybody's attention), would open up the belly of each animal and make comments about the lesions and the rationale of the experiments, would discuss the conclusions and implications, and would decide what would be the next steps. One of the recurring questions was that of the nature of the hypothalamic neurohumor(s) that would trigger the pituitary to secrete ACTH in response to stress and, in response to other exteroceptive stimuli, such as cold, to secrete TSH. That question was not answered until many years later.

This purely descriptive type of experimental medicine, in a classical approach to endocrinology, with removal of one endocrine gland or another, replacement therapy, combination of treatments, etc., was in the grand tradition of the European experimental and clinical medicine of the first half of the century. What was so appealing about it was its intellectual simplicity and the fact that it addressed itself to sociologically important diseases, which were apparently reproduced in the laboratory with great ease. Few, if not none at all, of these speculations have survived studies which were more extensive and critical than those of Selye's over the next 20 years or so. Many of the otherwise ingenious experimental designs or protocols of Selye made use of experimental conditions that were so extreme as to bear no relationship or relevance, not only to physiology, but also to the cause of those diseases of man that they proposed to investigate and explain. But in the 1940s and 1950s, Selye was one of the major ferments of modern endocrinology. He was the source of many ideas which, whether accepted or, more often, challenged; whether confirmed as such or eventually profoundly modified, were at the roots of modern neuroendocrinology – what we now understand to be the mechanisms of regulation of salt and water and the role and wide use of corticoid therapy.

Selye was a man of great Viennese charm, the best dancing partner my wife claims to have had, a linguist who spoke quite fluently five languages, and an investigator who never worked less than 12 hours a day, 7 days a week to the end of his life, regardless of ill health in his last years. Even though our ways of thinking and approaches in the laboratory diverged very early on, I have never forgotten that it was to Selye that I owed the introduction to my life in experimental medicine. The passing of Selye is the passing of a valiant era of modern medicine. The astounding successes of the ultimate reductionism that is molecular biology are begging now for another Selye to move and integrate them, even if temporarily, into what he once called supramolecular biology.

A giant of biology

by C. Fortier

Department of Physiology, Laval University, Laval (Quebec, Canada)

Hans Selye is one of the rare giants of contemporary biology. Gifted with intuition amounting to genius, a remarkable capacity for the synthesis of ideas, and a prodigious amount of energy, he spent the last 40 years of his life in unceasing exploration of the vast field of nonspecific disease and aging. Thanks to the acuity of his

observation, the originality of his thought and the breadth of his vision, subjected to a rigorous experimental approach, he opened up entirely new perspectives on the interrelationships between man and his surroundings, and on the mechanisms involved in the adaptation of the organism to changes in the environment.

Within this enormous area, successive themes were worked out in detail: new concepts of stress, of the general adaptive syndrome and of diseases of adaptation; studies of calciphylaxy, thrombo-hemorrhagic phenomena, heart disease with multiple causation, and catatoxic steroids. These are only the major themes of a long life of research, and all of them had as their point of departure Selye's main thesis, that the nonspecific reactions of tissues against aggression are to a very large extent conditioned by humoral factors which can be analyzed, identified and, up to a point, deliberately influenced.

The audacity and originality of Selye's ideas – abounding at numerous conferences, and in his books and papers – may have repelled some of the more orthodox and traditional members of the scientific community. Still, they have inspired a great many others to follow his example, and take part in a fascinating exploration, which in beginning to disclose the genesis of one of the most important chapters of physiopathology and the development of a new pharmacology.

A constellation of young research workers formed around Selye who were attracted by the joys and difficulties of experimental science. They found in him a demanding master, but one who was anxious to share his insatiable intellectual curiosity with them, and to teach them how to compel nature to reveal its secrets. All his pupils – of whom I am one – were profoundly influenced by that experience, and many of them have distinguished themselves in their turn by opening up new fields of exploration.

On the stress of working with Dr Selye

by G. Gabbiani

Department of Pathology, University of Geneva, CH-1211 Geneva 4 (Switzerland)

The main qualification I have for writing about my experience at the Institute for Experimental Medicine and Surgery (IMCE), University of Montréal, is a certain degree of adaptation to stress, since I have survived working with Dr Selye almost every day (Dr Selye worked *every* day! ...), for about 8 years, one of the longest periods as far as his academic coworkers are concerned. This long-lasting contact has profoundly influenced my approach toward science and life in general, particularly if one takes into account that I started to work at the IMCE as a young graduate student, just after finishing medical school. Dr Selye liked to teach graduate students and to discuss not only experimental work, but also general philosophical problems. If I look back to the years of our collaboration, what I remember most vividly are these general discussions. All Dr Selye's students and coworkers have been greatly influenced in one way or another by his style of work and his approach toward science and life. As far as I am concerned, Dr Selye's strongest characteristics were: a rigid sense of discipline in the organization of laboratory life and of the experimental work, a sense of 'grandeur' applied to every initiative whether scientific or administrative, and finally the constant habit of analyzing the possible general implications of every new observation. Hopefully, the following episodes will illustrate these general attitudes.

I acutely experienced Dr Selye's rigidity concerning the organization of laboratory life at the end of my first year at the IMCE when I was responsible for the technical personnel working on Dr Selye's experiments. I wanted to go back to Italy for my holidays to visit my family to whom I had already announced my intention to stay for 4 weeks. In addition, I wanted to spend a week in Paris to visit a young technician with whom I was working in the laboratory and who became my wife a year later. However, I was allowed only 4 weeks vacation. Having worked practically every day of the year, I felt that I could take an additional week of holiday, and since Dr Selye was absent for an extensive conference tour, I approached one of his senior coworkers and explained to him my problem and my request. He was very sympathetic and told me that he would speak with 'the boss' as soon as he came back, and that I should not worry. I left Montreal, had beautiful holidays in Italy and in Paris, but when I was back in Montreal, Dr Selye did not speak to me for a few weeks and, to my great surprise, I did not receive a prize which was given every year to a promising young research assistant at the IMCE, despite the fact that Dr Selye had already mentioned to me that I would win this prize. This was important to me not only for psychological reasons, but also because I relied on this prize in order to buy a car which would be very useful during the cold Canadian winter. Fortunately for me, the prize was assigned by the Committee to my best friend who, very generously, lent to me the entire amount, thus helping me both practically and psychologically. Eventually, I started again to communicate with Dr Selye, but our relations came back to normal only a few months after.

Dr Selye's sense of 'grandeur' is illustrated by the following episode which took place just at the end of my second year at the IMCE, when I came back from Europe where I got married (for these holidays, I was absent precisely four weeks ...). Before leaving Montreal, Dr Selye had offered to me the position of 'First Assistant' at the IMCE. I was proud of this offer since I knew that Dr Selye relied on his First Assistant for both scientific and administrative problems. When I arrived at the institute, I went to the great Dr Selye in his office and after speaking about my wedding, the vacation and the latest results of the experiments, I expressed the intention to leave. To my surprise, Dr Selye said that he wished to accompany

me since, for a reason that I could not understand very well, he had been obliged to change the location of my office. We continued our conversation through the corridor and when he opened with a master key the door of my new office, I had the impression of returning to the Christmas days of childhood when I received presents from my parents. The office was a large room with a wall to wall carpet, beautiful furniture, and a nice painting on the wall in front of my desk. This was definitively unexpected and disproportionate to my new position. Indeed, I never managed since to have an office comparable to the one that I occupied between the age of 29 and 32 years. Although I have, hopefully, several years in front of me before retiring, I am sure (as far as a scientist can be) that I will never occupy an office of a similar standing. Episodes illustrating Dr Selye's constant attitude of analyzing the possible general implications of every new observation are best illustrated by his extraordinary productivity of exciting and innovative concepts. I am sure that I represent the overwhelming majority of his coworkers when I say that my stay at the IMCE was very hard but exciting and constructive. Dr Selye was for all of us a true Magister.

My years with Selye

by A. Robert

Diabetes and Gastrointestinal Diseases Research, The Upjohn Company, Kalamazoo (MI 49001, USA)

I have vivid memories of my years with Hans Selye (1950–1955). Fresh from medical school and residency, I was entertaining the dream of working for a year with this extraordinary man who had taught us experimental endocrinology in the second year of medical studies. His calm assurance and the clarity of his teaching had always stayed with me. It was during one of these lectures that he said that a good professor is one who tells what he has seen, not what he has read. The impact was stronger because in French the statement rhymed. («Un bon professeur est celui qui enseigne ce qu'il a vu, non pas ce qu'il a lu.») To know that he was actively engaged in pioneering research, that he was writing paper after paper and book after book, and that all this was happening only three floors above the lecture hall where he was teaching, had exerted a strong fascination on me.

I went to see him with trepidation, knowing that the eight to ten graduate students and post-docs who worked with him came from all over the world and had outstanding credentials. As for me, I had yet to see a laboratory setting, let alone a laboratory animal. There was an aura of solemnity and intensity in his institute. I could feel this just by walking the corridors. His office was a large carpeted room where everything was in perfect order. The walls were covered with books and a few art objects. There were four photographs: Arthur Biedl, Selye's major professor in Prague, Walter B. Cannon, whose work on adaptive reactions was very influential to Selye's scientific thinking, Claude Bernard, whose precepts on how to conduct experiments were highly valued by Selye, and Louis Pasteur, whom he regarded as the greatest scientist. He sat at a rather small desk. On both sides of it were what I was to learn later was his 'carousel'. These were round, rotating shelves containing loose-leaf books, in which the subject index to the world literature on endocrinology and stress was compiled. He had developed a logical system for classifying scientific papers, and was maintaining it himself by longhand writing.

After I expressed my desire to work in his institute, he explained to me how he viewed graduate students. He indicated that he would give me a research project and that my responsibility would be to read on the subject matter and learn the techniques. 'If, for instance, you need to measure the glutathione content of tissues, you will have to find how to do it yourself.'

What I intended to be one year became five. Those were the most formative years of my life. I soon became addicted to the experimental process, and decided that I would not go back to clinical medicine.

The major impact of Selye that most of us felt derived from these unique characteristics: he taught us to be personally involved in every step of our experiments, to develop a keen sense of observation that would allow us to see the unexpected, and above all he taught us by example.

Selye lived in the laboratory, doing surgery himself, handling animals, looking at histological slides, and writing profusely. Unlike so many of us, then and later, he would not put data in a drawer for future use. Once an experiment was completed, its fate was to become a manuscript. His extraordinary sense of observation, his intuition and his near totally open mind led him to unexpected discoveries that were much more startling than those derived strictly from logical planning. Several of his most remarkable discoveries were, in his own admission, due to chance, the kind of chance that 'only favors the prepared mind', as Pasteur had said. For instance, the discovery of the 'alarm reaction' in 1936 derived from the incidental finding that rats became sick when given an impure ovarian extract. It required an active and observant mind to connect such seemingly unrelated changes as an increase in adrenal size, a decrease in thymus and spleen size, and presence of gastric ulcers. He connected these three observations and synthesized, so to speak, the syndrome of the 'alarm reaction'. His discovery of the anesthetic property of steroids came about when he was told by a technician that progesterone was toxic to rats. Soon after injection, they would go to sleep. Instead of disregarding such a surprising effect, and, as many of us would have done, order a different batch of progesterone, he went with the technician, asked her to repeat in front of him what she had seen, and indeed the same phenomenon happened. Within five minutes after injecting progesterone, the rats were asleep. Selye noted that she was injecting the steroid i.p., instead of s.c. as she was supposed to do. As was so characteristic of him, he realized

the unusualness and potential importance of this observation, and spent much of the next two to three years studying this new property not only of progesterone, but of a variety of steroids, the anesthetic effect. Another observation was that of the granuloma pouch technique. Air can be injected under the skin of rats and then an irritant introduced into the air cavity. Within a few days, an inflammatory reaction develops all around the air sac, with formation of an exudate into the cavity. This technique has since been extensively used for the study of inflammation. Again, it started with a chance observation, One day, he was injecting air into the peritoneal cavity to produce a pneumoperitoneum for some purpose that I forgot. In one of these animals the needle accidentally slipped and some of the air was injected s.c. Unlike the skin of several species, that of the rat is so flexible that the air, instead of diffusing haphazardly, separates the skin from the s.c. tissue and forms a well delineated air pocket. Since he was working at the time on inflammatory reactions, his active mind thought of exploiting this peculiarity by injecting irritants into the air pocket.

He was the best of teachers, not only because of the clarity of this thinking but also because that he was doing exactly what he was preaching. A hard worker, coming to the lab before dawn and leaving after dusk, he used every minute of his time productively. Yet, I never saw him hurried or impatient. I often thought that he was calm and poised primarily because he was so well organized. One particular Sunday afternoon in July 1953, a bright sunny warm day, I happened to be in the lab. At one point, he came out of his office and seeing me he came and said: 'One has to be crazy to be indoors in a lab on a day like this.' He then added with a twinkling in his eyes: 'But only the crazy ones achieve something in life.'

Whatever I was able to achieve myself, I owe it to him.

The creative and productive life of Hans Selye: a review of his major scientific discoveries

by S. Szabo

Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston (MA 02115, USA)

It is difficult to review the accomplishments of a scientist and a physician who has written or co-authored more than 1700 publications and compiled 40 volumes of books. His major contributions nevertheless may be divided into four groups as presented in tables 1–4. Although the topics are diverse, the common thread is the presence of hormones, especially steroids and stress in all the subjects of his investigations. In this review emphasis is placed on his discoveries as described in scientific journals (an additional appraisal has been prepared based on books he has written or edited).

Stress and steroids

In the first group of discoveries related to stress and steroids (table 1), a short report published in 1936 in 'Nature' is really historic[7]. The title 'A syndrome produced by diverse nocuous agents' implied a new unifying concept in analyzing and classifying tissue injury and diseases. First called the General Adaptation Syndrome, it represented the foundation of *biologic* stress research as opposed to the more colloquial physical stress[18,21]. He recognized 'the significance of the adrenals for adaptation'[8] and proved the importance of the pituitary-adrenocortical axis in stress reaction very early during his investigations[8,15,18,21]. He classified the steroids and named the products of the adrenal cortex *corticoids* and subdivided them into glucocorticoids and mineralocorticoids[10,13,14]. Elegant discussion of this classification is presented in his once very popular textbook[19]. He discovered the anesthetic action of steroids as well[11] ...

The functional and structural alterations in the adrenal gland during alarm reaction were described in his first reports which also formulated the 'triad' of stress, i.e., adrenal enlargement, thymo-splenic atrophy and gastric ulcers[7,8,18,21,24]. The specificity of these changes in their manifestation and their nonspecificity in origin were emphasized starting from the early descriptions. Namely, *stressors* diverse in nature (e.g. cold, heat, restraining immobilization, toxic chemicals, severe infections) caused the stereotyped *stress* response which, before his 1936 article, were almost uniformly accepted as specific manifestations of these agents. Although the wording of the definition of stress changed during the years, the meaning was always identical: stress is the nonspecific responses of the body to any demand made upon it[38,39]. About 40 years after the first description of stress syndrome, Selye introduced the distinction in perceiving stress as unpleasant (distress) and pleasant (eustress) reactions[38–40]. He, like Levi[6], emphasized that most of the endocrine responses (e.g., ACTH, corticoids secretion) were identical in the two reactions[38–40].

Although Selye was a pioneer advocating the importance of the adrenal *cortex* in stress, he accepted the role of the adrenal *medulla* as well. He apparently had a good working relationship with Cannon who characterized the importance of the sympathetic nervous system and the secretion of medullary catecholamines in 'emergency

Table 1. Stress and steroids

Topics	Initial key publications Journal, year	References
1) Stress and general adaptation syndrom	Nature, 1936; J. clin. Endocr., 1946	7, 18
2) Significance of adrenal cortex for adaptation	Science, 1937; 1955	8, 24
3) Classification of steroid (gluco- and mineralocorticoids, luteoids, folliculoids, testoids)	Nature, 1941, 1943; Endocrinology, 1942	10, 13, 14
4) Anesthetic effect of steroids	Am. J. Physiol, 1941	11
5) Distress *vs* eustress	Can. med. Ass. J., 1976	40

reaction' and during the 'fight or flight' syndrome. However, Selye did politely complain that Cannon did not see a role for the adrenal cortex and corticoids[41]. It is now accepted that the proximity of the adrenal cortex and medulla is not a casual coincidence and both have their independent and related contributions to the metabolic and functional changes during stress[1,2,39]. Namely, the rapid release of catecholamines with their short half-life and distinct effect on the early phase of adaptation (e.g. cardiovascular system, metabolism) is coupled with a slightly delayed rise in corticoid secretion resulting in prolonged metabolic and structural alterations in several organs[21,39].

Steroids and inflammation

Some of Selye's major contributions were related to the effects of stress and steroids on inflammation (table 2). His pioneering experimental work on the pro- and antiinflammatory action of mineralo- and glucocorticoids preceded by several years[4,9,12,20,44-46,58] the human studies of Hench and coworkers who were awarded the Nobel prize for the application of glucocorticoids in rheumatic inflammatory disorders[5]. An additional benefit of Selye's early work with corticoids was the discovery of DOC-hypertension, periarteritis nodosa and rheumatoid arthritis animal models and the aggravating action of sodium chloride[3,12,16,52,54,58,60]. The first easily applicable method to quantitate the inflammatory exudate, the granuloma-pouch technique, was also introduced by him[22,25]. His extensive work on inflammation that he considered his 'permanent research subject'[41], was also sparkled with significant contributions concerning the role of mast cells in inflammation and various forms of 'anaphylactoid edema'[23,31,47,55].

Table 2. Steroid and inflammation

Topics	Initial key publications Journal, year	References
1) Effect of stress on inflammatory reactions	Am. J. Physiol., 1938; Lancet, 1940; Can. med. Ass. J., 1944	9, 15, 44
2) Discovery of anti- and proinflammatory action of gluco- and mineralocorticoids	Can. med. Ass. J., 1940, 1949; Archs Path., 1943; Endocrinology, 1946	4, 20, 45, 46, 52
3) Development and treatment of rheumatoid lesions	Can. med. Ass. J., 1943; Lancet, 1946; Rev. Can. Biol., 1950	3, 16, 58
4) Development of 'granuloma pouch' technique	Proc. Soc. exp. Biol. Med., 1953; Br. J. Cancer, 1957	22, 25
5) Contributions to the role of mast cells and 'anaphylactoid edema'	Circulations Res., 1954; Int. Archs Allergy, 1954; Brit. J. exp. Path., 1963; Science, 1966	23, 31, 47, 55

Table 3. Other related topics

Topics	Initial key publications Journal, year	References
1) Production of 'endocrine kidney'	Nature, 1946	17
2) Organ-specific calcifications and calciphylaxis	Science, 1961; Archs Path., 1962; Endocrinology, 1963, 1964	48, 49, 51, 63
3) Organ-specific hemorrhages and necrosis	Br. J. exp. Path. 1961, 1963; Science, 1965; Blood, 1965; Nature, 1965	29, 50, 47, 65, 66
4) Cross resistance, pluricausal diseases	Can. J. Biochem., 1961; Exp. med. Surg., 1966; Science, 1967	30, 32, 43
5) Catatoxic concept	Can. med. Ass. J., 1969; J. Pharmac. Sci., 1971	34, 36

Other related topics

The 'other related topics' are also associated with hormonal adaptation (table 3). With a simple surgical manipulation he transformed (in the middle of his DOC-hypertension-nephrosclerosis era) the kidney into a hormone (e.g. angiotensin)-producing organ[17]. It was not only coincidence but also his sharp 'lateral vision' that allowed him, while studying the hormonal modulation of localized inflammations, to discover calciphylaxis, a state of hypersensitivity to organ-specific calcifications[48,49,51,63]. A side-line of these studies was the observation that the localized tissue injuries were often hemorrhagic. Selye named these 'thrombohemorrhagic phenomena' which represented reactions similar to Swartzman phenomenon but involved organs other than the kidney[29,47,50,65,66]. Although the macroscopic and light microscopic changes of both organ-specific calcifications and hemorrhagic phenomena were meticulously described in several publications and monographs by Selye and coworkers, the underlying mechanisms remain poorly understood. Partly for this reason, the significance of even such stunning discoveries as the selective destruction of experimental tumors by hemorrhage or calcification is generally not appreciated today[62,64].

It was thus almost natural for Selye who was usually working with 'sensitizers', 'challengers' and 'modifiers' of disease processes, to recognize that most of the experimental and human disorders are pluricausal or multifactorial (table 3)[30,32]. Very often, the real or implicated etiologic agents were opposite to each other in action exhibiting 'cross resistance'[32,43]. These were revolutionary concepts at the time.

Stress and cardiovascular system

The pluricausal or multifactorial diseases are best exemplified by his work with models of cardiovascular diseases. The initial discoveries again originated from the work on

Table 4. Stress and cardiovascular system

Topics	Initial key publications Journal, year	References
1) Production of hypertension, cardiac hypertrophy and nephrosclerosis by DOC	Can. med. Ass. J., 1942, 1943; Am. Heart J., 1944	12, 53, 54
2) Aggravation of nephrosclerosis and experimental hypertension by sodium chloride	Proc. Soc. exp. Biol. Med., 1943; Am. Heart J., 1949	60, 61
3) Induction of infarctoid myocardial lesions (ESCN)	J. Pharmac. exp. Ther., 1958	59
4) Protection against experimental arteriosclerosis and ESCN by magnesium and potassium salts	Am. Heart J., 1958; Can. J. Biochem. Physiol. 1958; Am. J. Path, 1959	26, 42, 57
5) Protection against ESCN and digitoxin cardiotoxicity by amiloride and spironolactone	J. Am. med. Ass., 1968; Science, 1969	33, 56

steroids and inflammation, such as the induction of hypertension, cardiac hypertrophy and nephrosclerosis by DOC and other conditioning factors (table 4)[53,54,60]. In 1943 and 1949, much before the modern dietary craze, he discovered that sodium chloride aggravated the cardiovascular lesions[60,61]. He recognized that the combination of high sodium ingestion and synthetic mineralocorticoids followed by exposure to heavy stress (e.g. restraint) or high fat diet resulted in patchy myocardial lesions characterized as electrolyte-steroid-cardiopathy with necroses (ESCN)[59] which could be prevented by potassium salts[26,33,42,57]. He devoted more than 10 years of intensive work to this field and produced major advances in our understanding of the pathogenesis and hormonal sensitivity of cardiovascular lesions and for the first time pointed out the opposite effects of sodium and potassium in the alterations. Three monographs, the most recently published in 1970, summarize these contributions[35].

Epilogue

His last major achievements in experimental medicine were also related to steroids. He recognized an antitoxic action of certain steroids which he named catatoxic (à la *cata*bolism) properties[34,37]. A steroid, pregnenolone-16α-carbonitrile, discovered by him, has only catatoxic and no other hormonal effects, while spironolactone is also an antimineralocorticoid[36,37,56]. Some of these catatoxic actions are due to induction of hepatic cytochrome P-450 or other influences upon drug disposition[67,68]. Following this he prepared a new edition for his popular book on stress[27] and his major scientific treatise was also devoted to a contemporary overview of biologic stress[39].

His numerous students and coworkers all over the world like the famous 'Claude Bernard Visiting Professors' attest that he was a demanding man but a great scientist and teacher. He created a lot of distress in his environment, expected loyality and adaptability or ... Since his experimental work was heavily descriptive, often not analytical enough by modern criteria, his students often learned not only the creative aspects of research and how to explore those routes, but also what not to do after becoming independent ... Nevertheless, his endless emphasis on originality and critical approach to scientific inquiries[28] leave the impression of admiration and eustress in most of us.

1 Axelrod, J., and Weinshilboum, R., Catecholamines. N. Engl. J. Med. *287* (1972) 237–242.
2 Benson, H., The Relaxation Response. William Morrow Company, New York 1975.
3 Coutu, L.L., and Selye, H., Action du 21-acetoxy-pregnenolone (artisone) comparée à celle de l'acétate de désoxycorticostérone (DCA) et du composé E de Kendall (cortisone) sur l'arthrite expérimentale. Rev. Can. Biol. *9* (1950) 308.
4 Hall, C.E., Dontigny, P., Beland, E., and Selye, H., The role of the adrenals in the production of nephrosclerosis by anterior pituitary preparations. Endocrinology *38* (1946) 296–299.
5 Hench, P.S., Kendall, E.C., Slocumb, C.H., and Polley, H.F., The effect of a hormone of the adrenal cortex (17-hydroxy-11-dehydrocorticosterone: compound E) and of pituitary adrenocorticotropic hormone on rheumatoid arthritis; preliminary report. Proc. Staff Meet. Mayo Clin. *24* (1949) 277–297.
6 Levi, L. (ed.), Society, Stress and Diseases. Oxford University Press, New York 1971.
7 Selye, H., A syndrome produced by diverse nocuous agents. Nature *138* (1936) 32.
8 Selye, H., The significance of the adrenals for adaptation. Science *85* (1937) 247–248.
9 Selye, H., The prevention of adrenalin lung edema by the alarm reaction. Am. J. Physiol. *122* (1938) 347–351.
10 Selye, H., Pharmacological classification of steroid hormones. Nature *148* (1941) 84–85.
11 Selye, H., The anesthetic effect of steroid hormones. Am. J. Physiol. *133* (1941) 442.
12 Selye, H., Production of nephrosclerosis by overdosage with desoxycorticosterone acetate and sodium chloride. Can. med. Ass. J. *49* (1943) 88–92.
13 Selye, H., Fundamental rules regulating the actions of steroid hormones. Endocrinology *30* (1942) 1040.
14 Selye, H., An attempt at a natural classification of the steroids. Nature *151* (1943) 662–663.
15 Selye, H., Role of the hypophysis in the pathogenesis of the diseases of adaptation. Can. med. Ass. J. *50* (1944) 426–433.
16 Selye, H., Relation of the adrenal cortex to arthritis. Lancet *250* (1946) 942.
17 Selye, H., Transformation of the kidney into an exclusively endocrine organ. Nature *158* (1946) 131.
18 Selye, H., The general adaptation syndrome and the disease of adaptation. J. clin. Endocr. *6* (1946) 117–230.
19 Selye, H., Textbook of Endocrinology. Acta Inc., Med. Publ., Montreal, 1st edn 1947, 2nd edn 1949.
20 Selye, H., Effect of ACTH and cortisone upon an 'anaphylactoid reaction'. Can. med. Ass. J. *61* (1949) 553–556.
21 Selye, H., Stress. Acta Inc., Med. Publ., Montreal 1950.
22 Selye, H., Use of 'granuloma pouch' technic in the study of antiphylogistic corticoids. Proc. Soc. exp. Biol. *82* (1953) 328–333.
23 Selye, H., Sensitization of individual vascular territories to the antiphlogistic effect of hydrocortisone. An example of 'selective conditioning'. Circulation Res. *2* (1954) 53–59.
24 Selye, H., Stress and disease. Science *122* (1955) 625–631.
25 Selye, H., Effect of inflammation upon the growth of transplantable neoplasms as demonstrated by the 'double granuloma-pouch' technique. Br. J. Cancer *11* (1957) 550–553.
26 Selye, H., Production with sodium sulphates of an electrolyte-steroid-cardiopathy characterized by necrosis (ESCN) and its prevention by $MgCl_2$ and KCl. Can. J. Biochem. *36* (1958) 717–719.
27 Selye, H., The Stress of Life. McGraw Hill, New York, 1st edn 1959, 2nd edn 1976.
28 Selye, H., From Dream to Discovery. McGraw Hill, New York 1964.
29 Selye, H., Induced hypersensitivity to cold. Science *149* (1965) 201–202.
30 Selye, H., Pluricausal diseases. Exp. Med. Surg. *24* (1966) 191–209.
31 Selye, H., Mast cells and necrosis. Science *152* (1966) 1371–1372.
32 Selye, H., Ischemic necrosis: prevention by stress. Science *156* (1967) 1262–1263.
33 Selye, H., Prevention of cardiac necrosis by amiloride. J. Am. med. Ass. *206* (1968) 103–104.
34 Selye, H., Catatoxic steroids. Can. med. Ass. J. *101* (1969) 51.
35 Selye, H., Experimental Cardiovascular Diseases. Springer-Verlag, New York 1970.
36 Selye, H., Hormones and Resistance. J. pharm. Sci. *60* (1971) 1–28.
37 Selye, H., Hormones and Resistance. Springer-Verlag, New York 1971.
38 Selye, H., Stress Without Distress. Lippincott, Philadelphia 1974.
39 Selye, H., Stress in Health and Disease. Butterworth, Boston 1976.
40 Selye, H., Forty years of stress research: principal remaining problems and misconceptions. Can. med. Ass. J. *115* (1976) 53–56.
41 Selye, H., Personal communication.
42 Selye, H., and Bajusz, E., Sensitization by potassium deficiency for the production of myocardial necrosis by stress. Am. J. Path. *35* (1959) 525–535.
43 Selye, H., Bajusz, E., and Strebel, R., Cross-resistance to cardiotoxic agents. Can. J. Biochem. Physiol. *9* (1961) 519–525.
44 Selye, H., and Dosne, C., Treatment of wound shock with corticosterone. Lancet *2* (1940) 70–71.
45 Selye, H., Dosne, C., Bassett, L., and Whittaker, J., On the therapeutic value of adrenal cortical hormones in traumatic shock and allied conditions. Can. med. Ass. J. *43* (1940) 1–8.
46 Selye, H., Dosne, C., and Dalton, A.J., On the antagonism between the actions of adrenaline and adrenal cortical extracts. Can. med. Ass. J. *42* (1940) 188–190.
47 Selye, H., Gabbiani, G., and Tuchweber, B., The role of mastocytes in the regional fixation of blood-borne particles. Br. J. exp. Path. *44* (1963) 38–46.

48 Selye, H., Gabbiani, G., and Tuchweber, B., Protection by calciphylaxis against an otherwise fatal parathyroid-hormone intoxication. Endocrinology *73* (1964) 825–829.
49 Selye, H., Gentile, G., and Prioreschi, P., Cutaneous molt induced by calciphylaxis in the rat. Science *134* (1961) 1876–1877.
50 Selye, H., and Grasso, S., Chemical production of myocardial necrosis with vascular occlusion. Br. J. exp. Path. *42* (1961) 564–567.
51 Selye, H., Grasso, S., and Gentile, G., Selective uterine or pancreatic lesions by calciphylactic system. A.M.A. Archs Path. *74* (1962) 554–559.
52 Selye, H., and Hall, C.E., Pathologic changes induced in various species by overdosage with desoxycorticosterone. A.M.A. Archs Path. *36* (1943) 19–31.
53 Selye, H., and Hall, C.E., Production of nephrosclerosis and cardiac hypertrophy in the rat by desoxycorticosterone acetate overdosage. Am. Heart J. *27* (1944) 338–344.
54 Selye, H., Hall, C.E., and Rowley, E.M., Malignant hypertension produced by treatment with desoxycorticosterone acetate and sodium chloride. Can. med. Ass. J. *49* (1943) 88–92.
55 Selye, H., and Heuser, G., Experimental studies concerning the role of hormonal, vascular and nervous factors in the regulation of the 'anaphylactoid reaction' of the rat. Int. Archs Allergy *5* (1954) 52–62.
56 Selye, H., Krajny, M., and Savoie, L., Digitoxin poisoning: prevention by spironolactone. Science *164* (1969) 842–843.
57 Selye, H., and Mishra, R.K., Prevention of the 'phosphate-steroid-cardiopathy' by various electrolytes. Am. Heart J. *55* (1958) 163–173.
58 Selye, H., and Pentz, E.I., Pathogenetical correlations between periarteritis nodosa, renal hypertension and rheumatic lesions. Can. med. Ass. J. *49* (1943) 264–272.
59 Selye, H., Renaud, S., and Nadasdi, M., Ability of various steroids to produce 'infarctoid cardiopathy' in rats conditioned by NaH_2PO_4. J. Pharmac. exp. Ther. *122* (1958) 504–511.
60 Selye, H., and Stone, H., Role of sodium chloride in production of nephrosclerosis by steroids. Proc. Soc. exp. Biol. *52* (1943) 190–193.
61 Selye, H., Stone, H., Timiras, P.S., and Schaffenburg, C., Influence of sodium chloride upon the actions of desoxycorticosterone acetate. Am. Heart J. *37* (1949) 1009–1016.
62 Selye, H., and Tuchweber, B., Hemorrhagic tumor necrosis elicited by an experimentally induced form of hypersensitivity to cold. Oncologia *20* (1966) 1–7.
63 Selye, H., Tuchweber, B., and Gabbiani, G., Role of the parathyroids in the production of osteitis fibrosa and soft tissue calcification by lead acetate. Endocrinology *72* (1963) 782–786.
64 Selye, H., Tuchweber, B., and Gabbiani, G., Limitation of tumor growth by induced calcification. Oncologia *17* (1964) 161–165.
65 Selye, H., Tuchweber, B., and Gabbiani, G., Effect of heparin upon various forms of the thrombohemorrhagic phenomenon (THP). Blood *22* (1965) 553.
66 Selye, H., Tuchweber, B., and Rohan, P., Thromboses in large veins and pulmonary embolisms induced by catecholamines or serotonin. Nature *208* (1965) 900–901.
67 Solymoss, R., Werringloer, J., and Toth, S., The influence of pregnenolone-16α-carbonitrile on hepatic mixed-function oxygenases. Steroids *17* (1971) 427–433.
68 Szabo, S., Kourounakis, P., Selye, H., and Da Silva, O., Pharmacodynamic interactions among gluco-, mineralo-, glucomineralocorticoids, pregnenolone-16α-carbonitrile and various drugs. J. Pharmac. exp. Ther. *188* (1974) 45–54.

0014-4754/85/050564-04$1.50 + 0.20/0

A tribute to the pioneering contributions of Hans Selye: an appraisal through his books

by Y. Taché

Department of Medicine, University of California at Los Angeles, Center for Ulcer Research and Education, VA Wadsworth Medical Center, Los Angeles (CA 90073, USA)

Hans Selye was an exceptional man: exceptional for his intellect, for his astonishingly retentive and encyclopedic memory, for his curiosity, the breadth and depth of his interests and his energy in pursuing them.

His ability to absorb, recall, synthetize and interpret information with extraordinary efficiency combined with his perfectionism and the use of several languages can be appreciated in the 15 scientific monographs in 26 volumes (table 1) as well as throughout his tremendous productivity including a total of 40 books and over 1700 scientific articles to his credit. To master more efficiently monographs from the point of view of both the author and the reader, Selye developed in 1956 a Symbolic Shorthand System for Physiology and Medicine[5] and in 1965 an analytico-synthetic style. In essence, the Symbolic Shorthand System (SSS) divides medical and related sciences into 20 classes, and uses Latin- and Greek-based abbreviations to simplify note-taking from publications, classification and retrieval of information. The SSS, for example, is very useful to simplify the complex nomenclature of steroids[6]. The analytico-synthetic style, used in his last six monographs, attempts to facilitate fact-findings by strictly separating the analysis of publications which are listed objectively as brief abstracts, from the subjective author's evaluation and synthesis, which precede the classified collection of concise abstracts.

His first four monographs (table 1) up to 1950 were devoted either to endocrinology in general (cf. his textbook was *the* primary text of the epoch) or to steroid producing organs (e.g. ovary, adrenal). His subsequent six monographs, initiated with the much acclaimed 'Stress' and the ensuing 'Animal Reports of Stress', published between 1950 and 1956 as well as his last major book 20 years later (i.e. 1976) were devoted to his famous and revolutionary concept of stress. True encyclopedias, these treatises covered the countless avenues of experimental and clinical research on stress as well as the wide variety of medical, social and legal implications. For example, in *Stress in Health and Disease* (1976), Selye undertook the gigantic task of coordinating, compiling and giving a panoramic overview of stress based on 7543 references selected from the explosive growth of scientific literature (110,000 entries in 1975). It was his last attempt to deal as a single author with all aspects of this growing and complex subject 40 years after his original description in a brief letter to the editor published in 'Nature' on 4 July 1936: 'A syndrome produced by diverse nocuous agents'. Thus, during the last few years of his life, Selye concentrated his talent as a writer and editor on biologic stress again. The three volumes[2–4] of Selye's *Guide to Stress Research* (1981–1983) and the journal 'Stress' published quarterly by the Hans Selye Foundation (1980–

Table 1. Scientific monographs written by Hans Selye

Title	Year	Publisher	Pages	References
1) Encyclopedia of Endocrinology (Section 1). Classified index of steroid hormones and related compounds (4 vols)	1943	A.W.T. Franks Publ., Montreal	728	17040
2) Encyclopedia of Endocrinology (Section 2). The ovary (2 vols)	1946	Bond & Wright, Montreal		
3) Textbook of Endocrinology editions (1 and 2)	1947, 1949	Acta Inc., Montreal	912	–
4) On the Experimental Morphology of the Adrenal Cortex	1950	C.C. Thomas Publ., Springfield, Ill.	105	75
5) Stress	1950	Acta Inc., Montreal	1046	5500
6) Annual Reports on Stress (5 vols)	1951–6	Acta Inc., Montreal	500–800	5000–6000
7) The Chemical Prevention of Cardiac Necroses	1958	Ronald Press, New York	235	418
8) The Pluricausal Cardiopathies	1961	C.C. Thomas Publ., Springfield	438	513
9) Calciphylaxis	1962	Univ. Chicago Press, Chicago	552	997
10) The Mast Cells	1965	Butterworth, Washington	498	2268
11) Thrombohemorrhagic Phenomena	1966	C.C. Thomas Publ., Springfield	337	1300
12) Anaphylactoid Edema	1968	Warren Green, St Louis	318	1104
13) Experimental Cardiovascular Diseases (2 vols)	1970	Springer-Verlag, New York	1155	5652
14) Hormones and Resistance (2 vols)	1971	Springer-Verlag, New York	1140	4275
15) Stress in Health and Diseases	1976	Butterworth, Boston	1256	7543

Table 2. Books for the layman written by Selye

Title	Publisher	Year	Pages
1) The Story of the Adaptation Syndrome	Acta Inc., Montreal	1952	225
2) The Stress of Life, 1st edn	McGraw-Hill, New York	1956	324
3) From Dream to Discovery	McGraw-Hill, New York	1964	445
4) In Vivo	Liveright, New York	1967	168
5) Stress without Distress	Lippincott, New York	1974	171
6) The Stress of Life, 2nd edn	McGraw-Hill, New York	1976	515
7) The Stress of My Life: A Scientist's Memory	Van Nostrand Reinhold, New York	1979	267

1982) were directed and edited by Selye to cover stress research based on collaborative efforts of the most qualified experts in specific facets of stress. He was also a co-editor of a book on stress and cancer[7].

Stress and particularly the endocrine manifestations of exposure to stressors have always remained his principal areas of research interest as reviewed in his previously mentioned monographs on stress and 'Hormones and Resistance'. However, Selye's relentless curiosity and peripheral vision drove him to master other stress-related subjects for which in short periods of time he brought sustained and original contributions over a period of years. 'Chemical Prevention of Cardiac Necroses' (1958), 'Pluricausal Cardiopathies' (1961) and 'Experimental Cardiovascular Diseases' (1970) are monographs gathering published and unpublished observations of Selye's group together with a general survey of the relevant literature on cardiovascular diseases produced by concurrent action of various potential pathogens (diets, stressors, mineralocorticoids). These monographs also illustrate the concept of pluricausal diseases. In essence, many maladies are not due to a single cause but to a 'pathogenic situation' in which several potentially pathogenic agents must act concomitantly and will cause disease only under certain circumstances (e.g. in the presence of special conditioning factors). His other monographs on 'Calciphylaxis' (1962) and 'Thrombohemorrhagic Phenomena' (1966) further emphasize the pluricausal pathogenesis by reviewing evidence of calciphylaxis, calcergy or thrombohemorrhage elicited by challengers only after suitable sensitization which determines both the character and the localization of the lesions.

Selye was always concerned about gaining public understanding of his work and not restricting the diffusion of his findings and concepts only to the scientific community. From 1956 to 1981 he wrote several books, which have been translated into many languages, addressed especially to the general public explaining in non-esoteric terms the mechanisms and manifestations of stress, the concept of catatoxic and syntoxic hormones, and later the code of behavior he derived from his scientific findings. Finally in his autobiography, the Stress of My Life (1979), and in a series of interviews described[1] in 'La Sagesse du Stress' (1981), he was able to talk casually about himself, his closest concerns and the precepts that guided him throughout his self-disciplined life.

The Symposium Hans Selye held in Montreal one year after his passing was a first attempt to pay homage to one of the greatest creative scientists and prolific writers of our time. May the brilliant contributions that he bequeathed in his writings shine even brighter over the coming years.

1 Goupil, G., Hans Selye. La Sagesse du Stress. Les éditions Nouvelles optiques, Montreal 1981.
2 Selye, H. (ed.), Selye's Guide to Stress Research, vol. 1. Van Nostrand Reinhold Company, New York 1981.
3 Selye, H. (ed.), Selye's Guide to Stress Research, vol. 2. Van Nostrand Reinhold Company, New York 1983.
4 Selye, H. (ed.), Selye's Guide to Stress Research, vol. 3. Van Nostrand Reinhold Company, New York 1983.
5 Selye, H., and Ember, G., Symbolic Shorthand System (SSS) for Physiology and Medicine, 4th edn Paradis-Vincent Ltd, Montreal 1964 (1st edn 1956).
6 Selye, H., Szabo, S., Kourounakis, P., and Taché, Y., Tentative rules for the SSS designation of steroids, in: Advance in Steroids Biochemistry and Pharmacology, vol. 3, pp. 142–164. Eds M.H. Briggs and G.A. Christie. Academic Press, New York 1972.
7 Taché, J., Selye, H., and Day, S.B. (eds): Cancer, Stress, and Death. Plenum Medical Book Company, New York 1977.

0014-4754/85/050567-02$1.50 + 0.20/0

Protein phosphatases from the cytosolic compartment of human erythrocytes

I. Japundžić, V. Garović and M. Japundžić
Institute of Biochemistry and Institute of Histology, Faculty of Medicine, Belgrade (Yugoslavia)

Little is known about the regulation of red blood cell metabolism by phosphorylation/dephosphorylation control mechanism. Erythrocytes (E) have been known to contain multiple cAMP-dependent and cAMP-independent, membrane bound and cytosolic protein kinases and phosphate acceptor proteins. However, almost nothing is known about the corresponding protein phosphatases. The aim of the present study was to identify and follow the ontogenetic evolution, kinetic and molecular properties of casein phosphatase activity from the cytosolic compartment of erythrocytes taken from cord blood and the blood of adult humans.

We resolved by DEAE-cellulose chromatography in 105,000 × g supernatant of human cord blood E three types of casein phosphatase activities, designated E_1, E_2 and E_3 in order of elution from the column, and only two, E_1 and E_3 in E taken from adults. E_2 can be monitored in the cytosol of adult E only in the presence of 5 mM $CoCl_2$. After gel filtration (Sephadex G-200 or Ultragel ACA 34) E_1, E_2 and E_3 phosphatase separated as single peaks of M_r 330,000, 230,000 and 180,000, respectively.

The optimal pH of the enzymes was 5.8 without additions. The enzymes were differentially affected by $CoCl_2$ and $MnCl_2$ which stimulated E_2 and E_3 but did not affect E_1. $CoCl_2$ shifted the optimal pH of E_2 and E_3 phosphatase toward neutral, at pH 7.2 and 6.8 in neonatal and adult E, respectively. PPi most effectively inhibited all three enzymes both in neonatal and adult E. The enzyme activities can be differentiated also by substrate specificity and kinetic properties, which are subject to changes during ontogenesis.

Cultures of fetal human liver cells in hormone-supplemented serum-free medium

M. Salas-Prato, J.F. Tanguay, G.R. Grotendorst, D. Barnes and G.O. Samuel
Department of Medicine, University of Montreal, and Hôpital Notre-Dame, Montreal (Canada), and National Institutes of Health, Bethesda, Maryland, and Department of Biological Sciences, University of Pittsburgh, Pittsburgh (Pennsylvania, USA)

The liver has a central role in metabolism and detoxification as well as the ability to regenerate. Due to these characteristics, there has been great interest in culturing liver epithelial cells. Cell culture systems provide models in which a particular organ or cell type can be isolated to study the hormonal factors affecting growth, function and morphology. However, most of the liver cellular cultures presently available utilize fetal bovine serum (FBS). As previously reported, we devised together with Sato, a hormone containing serum-free medium for culturing normal fetal mouse and human hepatoma cells consisting of a combination of six [epidermal growth factor (EGF), glucagon, cortisol, selenium, ethanolamine and cholera toxin] or seven factors (6+0.5% dialyzed FBS). We have already studied the effect of these factors on the growth, morphology and attachment of mouse fetal liver cells. Serum-free systems provide us with cultures which the exact composition of every component in the medium is known and in which one can study the effect of individual hormones, growth factors and others. In this communication, we report the culturing of human fetal liver cells (primary cultures) in hormonally-supplemented serum-free medium. The livers of 12–15-week-old human fetuses (obtained from therapeutic abortions) were utilized and the primary culture was done in a similar way as that done with fetal mouse. The cells dissociated in the six factors and various attachment factors [collagen, laminin, serum spreading factor (SSF) and fibronectin, CIG] were employed. The results obtained with the attachment factors indicate that these factors may affect attachment as well as growth and possibly function. All of these factors are more or less effective for attachment. Collagen and fibronectin appear to be more effective than the other factor in stimulating growth. To study the effect of the seven factors on growth, a series of experiments was done using collagen-coated dishes. As compared with the cells grown with no additions, there was virtually a doubling of cell number after culturing of cells with the seven factors. We are now in the process of examining how these factors affect morphology, function and differentiation.

The cytoskeleton of vascular smooth muscle cells in vivo and in vitro

G. Gabbiani, O. Kocher and W.S. Bloom
Department of Pathology, University of Geneva, CH-1211 Geneva 4 (Switzerland)

The remodeling of actin (A), vimentin (V) and desmin (D) in vascular smooth muscle cells (VSMC) of the rat thoracic aorta after mechanical endothelial injury, or when cultured in the presence of 10% FCS, was studied by means of immunofluorescence, densitometric analysis of SDS-polyacrylamide gels and bidimensional gels. In normal aortic media, almost all cells contained V and 50% of them contained D as well. VSMC were predominantly in the α-actin isoform on bidimensional gels. 15 days after endothelial injury, the VSMC which had migrated into the intima contained increased amounts of V and decreased amounts of A and D; moreover, β-actin isoform predominated, γ-actin increased and α-actin decreased. 75 days after injury, the endothelium had completely regenerated, and almost all the changes in VSMC in the intima had regressed. VSMC cultured following enzymatic digestion became confluent 10–13 days after plating; at this time their content in V increased and their content in A and D decreased compared to in situ or freshly isolated VSMC; D tended to disappear at later times. On bidimensional gels, β-actin became the predominant actin isotype; and γ-actin increased whereas α-actin decreased. The cytoskeletal composition of human atheromatous plaques is similar to that of VSMC in intimal thickening 15 days after endothelial injury and of replicating cultured VSMC. Hence, VSMC of intimal thickening early after mechanical injury of the endothelium and replicating cultured VSMC represent reliable experimental models of VSMC cytoskeletal changes during atheroma. (Supported by the Swiss National Science Foundation, grant No. 3.178-.0.82.)

Nucleolar organizer or disorganized nucleolus

P. Jean, P.-E. Messier and C.-L. Richer
Department of Anatomy, Faculty of Medicine, University of Montreal, Montreal (Quebec, Canada H3C 3J7)

The genes which transcribe 18S and 28S rRNAs are responsible for the formation of the nucleolus in interphase. In man, rDNA 18S and 28S is localized at the level of the secondary constrictions of the acrocentric chromosomes (Henderson et al., Proc. natn. Acad. Sci. USA *69* (1972) 3394). These nucleolar organizer regions (NOR) can be visualized by means of the silver-staining of the material of a protein nature to which they remain attached during mitosis. As an alternative to the use of ammoniacal silver solutions as in the classical techniques of Howell et al. (Experientia *31* (1975) 260) and of Goodpasture and Bloom (Chromosoma *53* (1975) 37), human lymphocytes in mitosis were stained using an aqueous solution with 50% of $AgNO_3$ for periods between 1 and 17 h. Analysis of prophase and metaphase chromosomes, using light or electron microscopy, shows that there is a silver-staining substance in the area of the NOR; this substance is most probably of nuclear origin, and its quantity dimin-

ishes gradually as mitosis progresses. These results confirm that the silver-staining technique reveals in the mitotic chromosome the product of transcriptional activity which took place during the preceding interphase. This product corresponds to vestiges of nucleolar fragments which disaggregate during mitosis. Thus, what one sees when one looks at an NOR is not so much a nucleolar organizer as a disorganized nucleolus.

(This work was carried out with a grant from the 'Comité d'Attribution des Fonds Internes de Recherche' of the University of Montréal.)

High-resolution analysis of human prophase chromosomes with R-band staining

C.-L. Richer, R. Drouin, M. Murer-Orlando and P. Jean
Department of Anatomy, University of Montréal, and Hôpital Ste-Justine, Montreal (Quebec, Canada H3T1C5)

Soon after 1956, when the exact number of human chromosomes had been determined, it became possible to account for a number of conditions, like Turner's and Klinefelter's syndromes, which had hitherto been considered to be entirely within the province of endocrinology. In 1971, when methods became available for the staining of chromosomes, research on anomalies of structure could be carried out in the light of our knowledge of the 300 bands which had been described at that time. However, the diagnostic ability of cytogenetics increased enormously when cell synchronization made it possible to prepare chromosomes which, in prophase, showed almost three times as many bands (about 850 per haploid set).

We have stained the long chromosomes obtained after synchronization with an antimitotic substance, amethopterin, using an R-banding method (RHG) which involves heat treatment followed by Giemsa staining. This adaptation of a method originally described for metaphase chromosomes has proved to be excellent for prophase chromosomes, and is much less inconvenient than other types of R-band staining.

We have made drawings which are as accurate a representation as possible of each chromosome with bands stained by the RHG method; these drawings are based on meticulous examination of the chromosomes of 20 phenotypically normal individuals. The new cytogenetic nomenclature has been adapted for this idiogram.

A comparative analysis of the bands in prophase and metaphase revealed certain mechanisms of organization of the R-bands, and some details of chromosome dynamics. In addition, the idiogram will be a useful tool in gene mapping, and it could also make the analysis of chromosome abnormalities easier, because such abnormalities (and even the newly discovered chromosomesyndromes, like the Prader-Willi syndrome, retinoblastoma, and aniridia with Wilms' tumor) very often involve R-bands.

(This work was carried out with a grant from the CRM.)

Autoantibodies to cytoskeletal structures in connective tissue disease (CTD)

J.L. Senecal, J.M. Oliver and N.F. Rothfield
Departments of Medicine and Physiology, University of Connecticut School of Medicine, Farmington (Connecticut, USA)

The cytoskeleton of mammalian cells consists of three fibrous systems: microtubules (MT), microfilaments (MF) and intermediate filaments (IF). Anticytoskeletal antibodies (A-CY) were studied in 103 connective tissue diseases (CTD) patients: 35 scleroderma (SCL), 32 systemic lupus erythematosus (SLE), 22 rheumatoid arthritis (RA), 14 polydermatomyositis (PDM), and in 40 normals using indirect immunofluorescence and freshly grown PTK2 cells as substrate. Anti-IF were found in high titers (> 1:100) in eight (57%) PDSM, seven (31%) RA, 10 (29%) SCL and six (19%) SLE patients, and in two (5%) normals. Anti-MF were found in high titers (> 1:50) in two (14%) PDM, five (14%) SCL, three (9%) SLE and one (4%) RA patients, and in none of the normals. Anti-IF were more common in CTD than in normals ($p < 0.01$). Anti-MF were IgG in six and IgM in five whereas anti-IF were IgM in 29 and IgG in two patients. Five years serial studies in five patients revealed the persistence of antibodies, with IgM to IgG switch in one patient. Only two patients had both anti-IF and MF. No anti-MT were found. An antibody that reacted with the spindle pole and midbody was found in one patient. A-CY, especially IgM anti-IF are common in CTD, occurring most frequently in PDM. The antibodies are present at disease onset and persist for prolonged periods after the disease has been treated.

Adenohypophysial hyperplasia

E. Horvath
Department of Pathology, St Michael's Hospital, University of Toronto, Toronto (Ontario, Canada MB5 1W8)

Immunocytochemistry and transmission electron microscopy demonstrate five distinct cell types in the human anterior pituitary: somatotrophs, lactotrophs, corticotrophs and gonadotrophs. All five cell types can give rise to adenoma. The morphologic characteristics of pituitary adenomas are well defined. Information is limited, however, on the nonneoplastic accumulation of adenohypophysial cells. This process is reversible and is termed hyperplasia. Since adenohypophysial cells are unevenly distributed and their numbers vary considerably in the adenohypophysis, strict morphologic criteria are needed in diagnosis of adenohypophysial hyperplasia.

Our histologic and, in part, immunocytologic as well as electron microscopic studies, performed on 750 surgically-removed and 1200 autopsy-obtained human pituitaries, provide conclusive evidence that all five cell types can undergo hyperplasia. Adenohypophysial hyperplasia may be associated with clinical symptoms and biochemical abnormalities, and may be responsible for various endocrine diseases, such as acromegaly, the amenorrhea-galactorrhea syndrome or Cushing's disease. Hyperplasia may be focal, nodular or diffuse; it may be secondary to target gland failure or to hypothalamic stimulation of currently obscure causes. In the differential diagnosis between hyperplasia and adenoma, a careful morphologic study of the pituitary is crucial. Hyperplastic foci are interspersed with other cell types and retain a well preserved reticulin network. Hyperplastic areas have no distinct borders with no compression of neighboring adenohypophysial cells. Adenomas usually consist of a homogeneous cell population, their reticulin network is distorted or absent; they are sharply demarcated and are surrounded by compressed nontumorous adenohypophysial cells and a condensed reticulin fiber network. In some cases, it is not possible to conclusively distinguish nodular hyperplasias from adenomas.

Cytogenetical evidence of the neuroectodermal origin of the pituitary gland

M. Japundžić, V. Lačković and I. Japundžić
Institute of Histology, Faculty of Medicine, Visegradska 26, Belgrade (Yugoslavia)

The nature and the precise embryological origin of the pituitary cells remain largely unknown. This is mainly because the early developing pituitary gland is still relatively inaccessible to methods of cytology and cell biology. We, therefore, studied the effects which can be described in cytological and cytochemical terms of gene mutations on the phenotypic characteristics of the endocrine cell in the mutant rats produced in our laboratory. We found that in the mutant-bearing animals, there were a number of genes that affect morphogenetic behavior and phenotypic

expression of the anterior pituitary cells. These genes may be identified by their characteristic pattern of the pleiotropic effects. The most prominent among the mutants' pleiotropic cells are cells of the melanocytic and neuronal phenotype, especially numerous at the level of the pars intermedia. Concomitantly with endocrine activity, some of the glandular cells take on melanosynthetic activity and possibly become autosensitive or *self-target cells*.

These observations allow the following conclusions: 1) the mutation affects the cytodifferentiation of the pituitary gland in a dramatic way; 2) neuronal and melanocytic differentiation of the pituitary cells reflects the presence of neuroectodermal genotype, and consequently provides the crucial evidence for neuroectodermal origin of the pituitary cells; 3) the mutation of pituitary cells forms a unique developing system with a number of operationally useful phenotypic markers elucidating the role of genetic and epigenetic factors in promoting the expression and maintaining of various cell phenotypes.

Pathology of prolactin-producing pituitary adenomas

K. Kovacs
Department of Pathology, St Michael's Hospital, University of Toronto, Toronto (Ontario, Canada M4N 2X3)

During the last 12 years, 800 surgically-removed pituitary adenomas have been studied in our laboratory by histology, immunocytology and electron microscopy. Based on hormone content, ultrastructural features and cytogenesis, tumors were classified into distinct entities. Prolactin-containing adenomas represented the most frequently occurring type; they constituted 42% of the tumors. The adenomas capable of producing prolactin differed in their morphologic appearance and were subdivided into groups: 1) densely granulated prolactin cell adenomas; 2) sparsely granulated prolactin cell adenomas; 3) mixed growth hormone cell-prolactin cell adenomas; 4) acidophil stem cell adenomas; 5) mammosomatotroph cell adenomas; 6) plurihormonal adenomas with prolactin content. The value of immunocytology and electron microscopy is emphasized in the differential diagnosis of pituitary tumors and in assessing their endocrine activity, growth rate and therapeutic responsiveness. (This work was supported in part by the Medical Research Council of Canada (grant MA-6349), the National Cancer Institute, DHEW, USA (grant 1 RO1 Ca 21905-01) and the St Michael's Hospital Research Society.)

Antitumoral effect of progesterone in the mouse

C. D. Pasqualini and C. Lanari
Seccion Leucemia Experimental, Instituto de Investigaciones Hematologicas, Academia Nacional de Medicina, Buenos Aires (Argentina)

Many years ago, Lipschutz demonstrated that the continuous administration of estrogens to guinea pigs resulted in the development of disseminated fibromatogenesis. Progesterone and other steroid hormones inhibited the development of these tumors. On the basis of this antifibromatogenic effect of progesterone, Lanari treated patients with mediastinal fibromatosis, desmomas, paraneoplastic fibrosis and atherosclerosis with high and sustained doses of medroxyprogesterone acetate (MPA). This treatment proved to be very successful in most cases (Medicina (Buenos Aires) *36* (1976) 281; *39* (1979) 826). Consequently, we investigated the effect of MPA on the growth of a fibrosarcoma in the mouse. The tumor originated in a BALB/c mouse 9 months after s.c. implantation of a glass cylinder, by foreign body tumorigenesis, and was maintained by s.c. passages as a syngeneic serial line. A total of 146 2–4-month-old BALB)c mice of both sexes received s.c. transplants of the fibrosarcoma either as a solid fragment by trocar or as cellular suspensions, both averaging 10^4–10^5 cells, together with 1) 50 mg MPA (Depo-Provera, Upjohn) in 1 ml, 2) 1 ml of the excipient and 3) 1 ml of physiological saline. A significant decrease in tumor incidence was registered in the MPA-treated mice, 1) 37% (20/54) as compared with the controls, 2) 84% (31/37) and 3) 94% (52/55) MPA-treated animals which did not survive showed a significantly prolonged death latency of 1) 45 ± 6 days as compared with the controls, 2) 29 ± 2 and 3) 35 ± 4 days. The antitumoral effect of MPA was not observed when the hormone was given 3–4 days. This antitumoral effect of MPA was not observed when the hormone was given 3–4 days after tumor challenge. This local effect of MPA does not seem to be specific for fibrosarcoma since preliminary results with a lymphoid leukemia serially transplanted in BALB/c mice yielded 25% (3/12) tumor incidence as compared with 100% in both control groups. It is postulated that progesterone affects the angiogenesis which accompanies tumor growth.

The general adaptation syndrome and neuroendocrine regulation of the lifespan

P. S. Timiras
Department of Physiology/Anatomy, University of California, Berkeley (California 94720, USA)

The General Adaptation Syndrome in its triphasic course resembles, as noted by Selye, the life course of the individual. In the first period of life, adaptive capacity is not completely developed and mortality is high. As the organism grows, the capacity to adapt progressively strengthens and, when adulthood is attained, resistance to stress reaches a maximum. In the third period of life, resistance slowly declines until death. As for adaptation, neuroendocrine interactions play an important role in the progression through the stages of the lifespan. In growth, development and maturation, neural (e.g. neurotransmitters) and hormonal (e.g. hypothalamic and hypophyseal hormones) signals trigger and integrate the physiologic events that lead from one developmental stage to the next. In aging, a specific signal may program the last stage of the lifespan or, alternatively, aging and death may result from the cessation of the life program once reproduction is terminated and the continuation of the species assured. In either case, our current observations point to the key role of neural and endocrine factors. Possible mechanism(s) include among neural factors: brain neurotransmitter imbalance (e.g. relative constancy with aging of serotonergic systems versus relative vulnerability of catecholaminergic systems), and/or neuronal loss (e.g. loss of cholinergic neurons in discrete brain regions); among endocrine factors: alterations in metabolism and secretion of hypophyseal hormones (e.g. increased TSH polymorphism with aging) and/or changed responsiveness of target tissues (e.g. changes with aging in receptor number, lipid protein interactions in membranes). These observations have led to interventive measures which, by interfering with neuroendocrine signals (e.g. brain serotonin/thyroid function) not only delay growth and development but also postpone some of the physiologic changes (e.g. cessation of reproductive capability) associated with aging and prolong the length of the lifespan.

Immunoregulatory aspects of pituitary function

I. Berczi and E. Nagy
Department of Immunology, Faculty of Medicine, The University of Manitoba, Winnipeg (Manitoba, Canada R3E OW3)

Numerous investigations indicate that stress and neurohormonal mechanisms in general influence immune reactions both in animals and man. This influence is exerted in part through the pituitary-adrenal axis, corticosteroids being the ultimate modifiers of immune reactivity. Studies on the role of other pituitary hormones in immune reactions remained controversial until

recently. We found that the antibody response to sheep red blood cells (a thymus-dependent antigen) and to bacterial lipopolysaccharide (LPS, a thymus-independent antigen) was severely impaired in hypophysectomized (Hypox) rats. The secondary response was also inhibited significantly but not completely by Hypox. Treatment of intact rats with bromocriptine (BRC), which inhibits prolactin secretion, suppressed humoral immunity as much as did hypophysectomy. Antibody production in Hypox rats was restored completely by daily treatment with prolactin (PRL), growth hormone (GH), or even by placental lactogen (PL). Each of these hormones was capable of complete restoration and other pituitary hormones (follicle-stimulating hormone, leuteinizing hormone, thyroid-stimulating hormone) had no effect. Adrenocorticotropic hormone (ACTH) antagonized restoration. BRC-suppressed animals could also be restored by either PRL or GH treatment, and ACTH inhibited restoration. Studies on contact sensitivity to dinitrochlorobenzene (DNCB) yielded indentical results: Hypox, BRC and ACTH were inhibitory whereas GH, PRL and PL restored reactivity. Similar results were obtained in rats with adjuvant arthritis. These observations indicate that the pituitary gland has the capacity to regulate immune reactions, since it secretes both immunostimulatory (GH, PRL) and immunosuppressive (ACTH) hormones. Further studies are underway in this laboratory to elucidate the mechanism and significance of neurohormonal immunoregulation. (Supported by MRC and the Arthriritis Society of Canada.)

The heart as an endocrine organ: cardioregulins

M. Cantin and J. Genest
Clinical Research Institute of Montreal, Montreal (Quebec, Canada H2W 1R7)

It was known since 1956 that the atria of mammals are made up of cardiocytes containing a secretory-like endocrine structure characterized by a large number of secretory granules and a well developed Golgi complex. The same structures are also present in the ventricular cardiocytes of a large number of nonmammalian species. Acid extracts of rat and human atria and isolated secretory granules from rat atria induce in the bioassay rat an important diuresis and natriuresis. Impure and purified extracts have also a vasorelaxing effect in vitro on strips of arteries previously contracted by norepinephrine or by angiotensin II. Injection of the extract into bioassay rats releases a large quantity of cGMP in the urine. The relaxation of vascular contraction is also accompanied by increased cGMP levels in arteries. Exposure of renal cortical cells in culture to purified preparations of atrial extracts also induces a large increase of cGMP in the culture medium. Antibodies against semi-purified fractions, one with a low mol.wt ($\sim$ 5000 daltons) and the other with a high mol.wt ($\sim$ 10,000–15,000 daltons) and immunocytochemical methods (Sternberger's technique, protein A-gold technique), have helped to localize these peptides into all three types of secretory granules of right and left atrium of rat and man. The purification of three of these peptides, their amino acid content and their sequence are now at our disposal. It is likely that this family of peptides is related to the elusive, long sought natriuretic hormone (third factor), to the diuresis of water immersion and to a great number of hitherto poorly understood cardiovascular phenomena. For this new class of hormonal peptides of cardiac origin we propose the name: cardioregulins.

Functions and composition of blood platelets in relation to risk factors for coronary heart disease

S. Renaud, R. Morazain, E. Dumont*, F. Godsey*, C. Thevenon and M. Lagarde*
*INSERM, Unit 63, Lyon-Bron (France) and *Department of Nutrition, University of Montreal, (Montreal, Quebec, Canada H3C 3J7)*

Saturated fats appear to be the main predisposing factor for coronary heart disease (CHD). In addition to the well-known effect on cholesterol and atherosclerosis, saturated fats markedly predispose to thrombosis either venous or arterial, as shown by numerous animal studies. Our own work has indicated that this predisposing effect could be mostly through increasing: 1) the reactivity of platelets to thrombin-induced aggregation or 2) the clotting activity of platelets (PF_3), both resulting from changes in the platelet phospholipid fatty acids.

Comparing farmers from East and South of France, East and West of Scotland, South of England, and two Belgian regions, we observed that as in animals, platelet functions (aggregation to thrombin, PF_3) were markedly well related to the intake of saturated fatty acids, and inversely related to consumption of polyunsaturated fatty acids. This was further confirmed by 1–4-year diet modification in Moselle farmers. Dietary calcium and alcohol were also inversely related to platelet functions. These two diet components markedly inhibit the effect of saturated fats on platelet functions in animals. Moreover, multivariate analysis of the results in farmers show that platelet functions are related to the fatty acid composition of the platelet phospholipds. Finally, the fatty acid 20:3 $\omega 9$, of which the level in the platelet phospholipids is related to a high intake of saturated fats, seems top play a key role in regulating platelet functions, probably through the lipoxygenase pathway, as shown by enriching human platelet phospholipids with this fatty acid. Consequently, dietary fats and other risk or preventive factors, appear to influence platelet function in exactly the same way they are known to influence CHD.

Right ventricular infarction in experimental malignant hypertension

J. M. Rojo-Ortega, A. Fueyo, M. Martinez and B. Marin
Department of Pathology, National Institute of Silicosis, and Department of Physiology, University of Oviedo, Oviedo (Spain)

Although there have been various studies on the left ventricular infarction (LVI) in systemic hypertension, little is known about the association of systemic hypertension with the so-called right ventricular infarction (RVI) (combined right and left ventricular infarction). In 127 Wistar rats (5 months old), severe hypertension was induced by complete ligation of the aorta (Ao-lig HT) just above the left renal artery (Rojo-Ortega and Genest, 1968). In addition, 20 age-matched rats were sham operated and served as controls. 10 days after the operation, the blood pressure (BP) was measured directly from the right carotid artery and recorded with a Beckman 611 A polygraph. Subsequently, the animals were sacrificed. The heart, kidneys and adrenals were weighed and processed for routine histopathological studies. The heart was sectioned at different levels in order to evaluate the location and extension of the infarcts. All animals with ligation of the aorta showed right ventricular infarcts located predominantly in the posterolateral wall. Left ventricular infarction was also encountered in 85% of the rats. In 60% of the animals, transmural infarcts of the right ventricle were associated with infarcts of the posterior part of the interventricular septum and with focal injured areas of the left ventricle. The acute lesions predominated over the healing lesions. These animals showed normal or low BP levels. However, when the healing lesions in the right ventricle were numerous and the interventricular septum was not severely damaged, the animals remained hypertensive. The intramyocardial small arteries of the right ventricle had more frequent and severe fibrinoid necrosis than those of the left ventricle. Similar vascular lesions were observed in the right kidney. The adrenal medulla was markedly enlarged. The control group did not reveal any of the described pathological features. We conclude that Ao-lig HT is always associated with right ventricular infarction. When acute RVI is associated with infarcts of the posterior part of the inter-

ventricular septum, it appears to be a major determinant in the evolution of blood pressure from hypertensive to normotensive or shock blood pressure levels. Furthermore, the present report points out a reliable and reproducible model of RVI.

The prevention by β-adrenoagonists of the hamster hereditary cardiomyopathy

G. Jasmin and L. Proschek
Department of Pathology, Faculty of Medicine, University of Montreal, Montreal (Quebec, Canada H3C 3J7)

Isoproterenol (ISO) increases the contractility of heart muscle by promoting calcium uptake by cardiocytes. Overtreatment with this β-agonist causes myocardial hypertrophy, severe necrotic changes which can be prevented by D-600, a calcium slow channel blocker. The latter drug was shown for the first time in our laboratory to prevent the development of the hamster hereditary cardiomyopathy; similarly propranolol, a β-adrenoblocker, was almost as efficient in reducing the heart lesions.
Unexpectedly, administration of ISO resulted in a significant reduction in severity of both heart and skeletal muscle lesions in these myopathic animals. The drug was injected s.c. in doses ranging between 2 and 8 mg/kg b.wt during 3–4 weeks in 28–30-day-old animals. There was in early and sustained decrease of serum CK levels and at the same time a normalization of serum alkaline phosphatase low values. While the heart ventricles wet weight was significantly increased, ISO proved to be efficient in preventing the usual coagulation and calcific heart necrotic changes. These findings correlated well with a diminution of calcium content in both skeletal and heart tissues of myopathic treated animals. Terbutaline, a specific β-agonist, injected in a daily dosage of 40 mg/kg exerts a similar effect. The protection afforded by these two β-adrenoagonists may help to further understand the pathomechanism of the hamster disease. Both drugs influence microcirculation as well as adenyl cyclase activity which deteriorates in this hereditary polymyopathy. (Supported by the Medical Research Council of Canada and the Muscular Dystrophy Association of Canada.)

Our experiences with several months survival of calves with total artificial hearts

J. Vašků
Institute of Pathophysiology, Faculty of Medicine, Purkinje University, Brno (Czechoslovakia)

Our studies on the total artificial heart (TAH) began in 1975. Our most successful group of animals having a TAH consisted of 12 calves which survived from 31 to 175 days. Seven calves from this group survived over 100 days, five calves over 150 days. Our TAH was designed and constructed in our institute. We used primarily the polymethylmethacrylate device TNS-BRNO-II, with the driving diaphragm and valves made of polyurethane. In one calf, a device made totally of polyurethane was employed (TNS-BRNO-III). The volume of these devices was 100 ml. To control and drive the cardiac prosthesis, our own drivers Chirasist TN-3, or Chirasist TN-4, respectively, were used. Anticoagulation and antiaggregation therapy was used in all the calves. Our technical instruments safely kept for several months the recipient of the TAH in very good physiologic state, all basic functions being maintained within physiologic limits. The driving was secured by a pneumatic system, which enabled the systolic and diastolic excursions of the driving diaphragm. To prevent hydraulic shock on the inflow valves during systole, the s.c. 'modelling stepper' was used as a component of the control system of our artificial hearts. Thus, especially on the left-hand side, possible valve damage was reduced.
Technical complications which caused termination of the experiment were leakage of the diaphragm on the right-hand side in two cases, and in one instance, an accident outside of the laboratory. In one case, combined technical and biologic reason, namely the degradation of the TAH quick connector and an enormous growth, terminated the experiment. Eight other experiments ended due to pathophysiologic reasons (i.e. thromboembolic complications, hemorrhage, sepsis, and chronic pulmonary stenosis, originating during the TAH implantation).
Pathophysiological findings in all of our calves included gradual increase of the central venous pressure, with concomitant hepatomegaly and increased enzyme levels, as well as marked shifts in serum protein. Complete recovery of the monoaminergic nerve terminals in the atrial stumps, where the inflow ports of the TAH were sewn, was ascertained after several months of pumping. We proved that even the large body of a fast growing calf (calf No. 59 'OMAR') could be kept in good physiological shape for 173 days by a pumping device of 100 ml volume. This calf weighed 210 kg at the end of the experiment, thus being the heaviest animal on record with a TAH. Further experiments are in progress to study future clinical applications of the total artificial heart in human patients.

Stress today

R.Q. Pasqualini
Faculty of Medicine, University of Buenos Aires, Buenos Aires (Argentina)

Stress entered the biomedical world in 1936. It originated from the experimental research carried by Hans Selye as a completely new concept together with a new interpretation of previously observed facts. During half a century, Selye was entirely dedicated to basic research and his work inspired many investigators in different parts of the world. The extensive projections of his concept of stress gradually led to an operational orientation in an attempt to obtain immediate benefits for the improvement of human health. Selye himself embraced this postulation and never failed to mention it in his later publications, putting the finishing touch with the foundation of the International Institute of Stress. Neither did he disdain the philosophical speculations. The projection of stress into the operational field had in some cases a sound basis but in other cases the aim was merely circumstantial. In both cases it gave origin to a varied technology destined more to elude or avoid the causes of stress than to cure stress.
At the present time, the main risk of this operational projection is a deviation from the basic and clinical research aimed at discovering the intimate nature of stress. However, experimental research on stress continues to be done in many centers, but with such a wide scattering of data that it is difficult to coordinate or integrate the numerous lines of work. One way to solve this problem, and at the same time foresee what remains to be done, is to draw a scheme of the present state of research on stress. The aim of this paper is to attempt such a task, taking into account the following points: 1) a list of the well established facts, including stressors, the way they act and their conditioning factors; 2) a review of the elements which participate in the process of stress, in addition to the hypothalamic-hypophyseal-adrenal system and the corticoids, such as other hormones, neurotransmitters, hormones of the nervous system and digestive tract, enzymes, metals, etc.; 3) an evaluation of the technology which has not yet been sufficiently applied in stress research.
A plea is made to enhance basic and clinical research in relation to the diseases of adaptation which, as Selye maintained, are the principal targets of stress research, understanding as diseases of adaptation only those which would not have developed without the intervention of a failure in adaptation, that is, a deviation of the homeostasis, configuring an 'allostasis'. Finally the necessity to proceed with the biochemical and pharmacological investigations is emphasized, in an attempt to discover hormonal deriva-

tives or analogues capable of correcting at a cellular or molecular level, the pathological effects of stress and in this way prevent the development of irreversible diseases of adaptation.

Stress and cancer

P. J. Rosch
American Institute of Stress, Yonkers (New York 10703, USA)

Relationships between stress or certain emotional states and cancer have been suspected since the time of Galen. Anecdotal medical reports over the past two centuries and particularly in the past two decades strongly support this contention. However, the mechanism whereby such effects might be mediated have only been uncovered in the past few years due to advances in the emerging discipline of psychoneuroimmunology. Implicit in these observations is the suggestion that opposing emotional or behavioral factors may bolster immune defenses and aid in the prevention and/or treatment of malignancies. Recent research in this area will be reviewed.

Effects of stress on testicular function

R. Collu, E. Gibb and J. R. Ducharme
Research Unit on Reproductive and Developmental Biology, Pediatric Research Center, Hôpital Sainte-Justine, Montreal (Quebec, Canada H3T 1C5)

A growing body of evidence can be found in the literature indicating that stress, either physical or psychological, can inhibit plasma testosterone (T) levels in several animal species including man. Plasma luteinizing hormone (LH) values are usually normal or even increased, indicating that the effects of stress are mainly exerted at the testicular level.

During the last few years, we have performed a series of experiments in order to establish the mechanisms of the inhibitory effects of stress on rat testicular function. The results obtained have indicated that immobilization stress rapidly induces a phenomenon of testicular desensitization to gonadotropins as well as a post-cyclic blockade of T biosynthesis. More recently, we have demonstrated that catecholamines are at least partly responsible for the T inhibitory effects of stress by acting through a testicular β_2-adrenergic receptor. In addition, we have recently shown that arginine-vasopressin (AVP) is also involved, at least during the first 2 h of an immobilization stress, in the inhibition of plasma T levels. Indeed, AVP-deficient Brattleboro rats were found to be insensitive to the inhibitory effects exerted by 2 h of immobilization on plasma T levels and on in vitro T production by purified Leydig cells.

These results indicate that stress can antagonize the testicular hormonal function through a multifactorial mechanism directly inhibiting T biosynthesis.

Central nervous system regulation of gastric secretion and stress ulceration by neuropeptides

Y. Taché
Center for Ulcer Research and Education, VA Center, Los Angeles (California 90073, USA)

For several decades, clinical and experimental evidence have accumulated to suggest that the central nervous system plays an important role in the regulation of gastric secretion and ulceration. However the neurochemical mediators in the brain involved in regulating gastrointestinal functions are poorly known. Studies carried out in rats demonstrate that injection of bombesin (0.25–1 μg) or corticotropin-releasing factor (CRF 0.5–10 μg) either into the cerebrospinal fluid (CSF) at the level of the cisterna magna, lateral or 4th ventricle or directly into the brain parenchyma at the level of the lateral hypothalamus or paraventricular nucleus, inhibit dose-dependently gastric acid secretion. Thyrotropin-releasing hormone (0.3–3 μg, TRH), given into the CSF or specific brain sites, induces a marked stimulation of gastric acid and pepsin secretion. The CNS action of these peptides appears mediated through modulation of the autonomic nervous system, namely through the vagus (TRH and CRF). Intracisternal injection of bombesin or β-endorphin completely prevents restraint stress-induced gastric lesions, whereas intracisternal TRH elicits within 4 h the development of gastric lesions in nonstressed rats and further enhances stress ulceration. From these data it is speculated that some specific neuropeptides in the brain may have major implications in modulating gastric function under physiologic or pathologic conditions.

Cytoprotection by prostaglandins

A. Robert
Department of Experimental Sciences, The Upjohn Company, Kalamazoo (MI 49001, USA)

Certain prostaglandins inhibit gastric secretion and exert antiulcer activity. Several prostaglandins also exhibit another property called 'cytoprotection'. It is a property by which many prostaglandins protect the mucosa of the stomach and the intestine from becoming inflamed and necrotic, when this mucosa is exposed to noxious agents.

There are two types of cytoprotection: direct and adaptive. Direct cytoprotection develops after administration of prostaglandins, orally or parenterally, whereas adaptive cytoprotection is obtained by stimulation of prostaglandin formation by the body.

Direct cytoprotection. Administration of certain prostaglandins to rats prevents formation of gastric lesions (ulcers, necrosis of the mucosa) produced by nonsteroidal antiinflammatory compounds such as aspirin and indomethacin, and by strong irritants such as absolute ethanol, a strong acid (0.6 M HCl) or a strong base (0.2 M NaOH), a hypertonic solution (25% NaCl), bile salts, and even boiling water. Similarly, necrotic lesions of the small or large intestine leading to peritonitis are prevented by treatment with certain prostaglandins. Such lesions are produced by nonsteroidal antiinflammatory compounds, corticosteroids and certain antibiotics.

Adaptive cytoprotection. Oral administration of 'mild' irritants is also cytoprotective. Thus, 20% ethanol, 0.35 M HCl, 0.075 M NaOH, 4% NaCl, 5 mM taurocholate, which by themselves are innocuous for the rat stomach, prevent gastric damage caused by the necrotizing agents mentioned above (e.g. absolute ethanol, etc.). This effect appears to be mediated by endogenous release of cytoprotective prostaglandins by the stomach, since such protection is abolished by prior treatment with indomethacin, an inhibitor of prostaglandin synthesis, and since mild irritants increase prostaglandin generation by the gastric mucosa. Cytoprotective prostaglandins may have clinical applications in the treatment of gastritis, gastric and duodenal ulcer, and perhaps certain forms of inflammatory bowel disease.

Pathogenesis of duodenal ulceration: lessons from animal models

S. Szabo
Departments of Pathology, Brigham and Women's Hospital, and Harvard Medical School, Boston (Massachusetts 02115, USA)

About 10 years ago, while working on my Ph.D. thesis in Dr Selye's Institute, I noticed that some chemicals (e.g. acetanilide, aniline, propionitrile) produced in the rat solitary or double ('kissing') duodenal ulcers (DU) which were only occasionally accompanied by nonspecific gastric erosions. Subsequently, we found with Dr Selye that cysteamine was especially potent and specific in inducing DU that often perforated in 24 h after a

single dose. I also recognized a structure-activity correlation among these compounds and predicted the duodenal ulcerogenic property of other chemicals (e.g. n-butyronitrile, cystamine, 3,4-toluenedithiol), and developed models of chronic active DU. It became apparent that these acute and chronic DU might serve as animal models of the human disease where the preulcerogenic stage is virtually never accessible for investigations. The experimental and human DU are indeed similar by morphologic criteria (e.g. localization on anterior and posterior wall, tendency to perforate or penetrate into adjacent organs like pancreas, liver), functional alterations (frequently elevated gastric secretion and serum gastrin levels), and response to therapeutic interventions (e.g. antacids, antisecretory agents or vagotomy). Using the animal models of DU caused by cysteamine or propionitrile and biochemical, functional and morphologic methods in the rat we learned recently about the pathogenesis of duodenal ulceration that includes 1) the presence of acid (not necessarily in excess) in the proximal duodenum, 2) impaired neutralization of acid by duodenal mucosa and submucosa, 3) increased duodenal motility resulting in inefficient delivery of pancreatic and biliary bicarbonate from distal to proximal duodenum, leaving an unusually large amount of acid unneutralized in the duodenal bulb. The ulcer formation starts in the absorptive cells on the tip of the villi and progresses centrifugally. The localization of the ulcers on the anterior and posterior wall is due to the special configuration of the duodenum as fixed by ligaments and confined between adjacent organs. Ligamentectomy and mechanical fixation of the duodenum changed the location of the ulcers suggesting that there is no primary biochemical or blood flow defect on the two walls in the proximal duodenum. Multiplicity of pathogenetic factors associated with DU identical in site and shape is indicated by the recent finding that only the cysteamine and not the propionitrile-type of DU is accompanied by multiorgan somatostatin depletion. In addition to the involvement of acetylcholine, histamine and gastrin in experimental DU, we recently suggested a putative role for dopamine and sulfhydryls in duodenal ulceration. Thus, the use of animal models allowed not only a reconstruction for the first time of the pathogenesis of DU starting from biochemical preulcerogenic changes to ulcer and healing stages, but revealed new biochemical mediators and modulators that might be amenable to modulation for investigational and therapeutic purposes.

The control of the catalytic cycle of liver microsomal cytochrome P-450

J. Werringloer
Department of Toxicology, University of Tübingen, Tübingen (Federal Republic of Germany)

Cytochrome P-450, a family of unique hemeproteins embedded in the lipid domain of the endoplasmic reticulum of many cell types, plays a key role in the detoxification and toxication of many drugs and environmental chemicals as well as in the metabolism of certain endogenous substances such as steroids and fatty acids. The activity of this enzyme system varies greatly depending upon the exposure of the living organism to conditions resulting in changes of the total concentration of cytochrome P-450 as well as in an alteration of the composition of its various isoenzymes due to selective induction and/or suppression of enzyme synthesis. Under any given condition, however, the monooxygenase activity of cytochrome P-450 associated with the hepatic endoplasmic reticulum has been recognized to be controlled by the velocity of the electron transfer reactions required for the activation of molecular oxygen. These electron transfer reactions resulting in the stepwise reduction first of the ferric hemeprotein-substrate complex and second of the ternary complex of cytochrome P-450 formed upon the additional binding of molecular oxygen were found to be regulated differentially in an inverse pH-dependent manner termed 'Counter-poise'-regulation; i.e., the transfer of the first electron to high spin ferric cytochrome P-450 could be shown to be facilitated with increasing pH as a function of the catalytic activity of the NADPH-cytochrome P-450 reductase, whereas the transfer of the second electron to oxygenated cytochrome P-450, a reaction catalyzed by both the NADPH-cytochrome P-450 reductase and cytochrome b_5, was found to be enhanced with decreasing pH presumably as a consequence of an attenuation of the negativity of the redox potential of oxy-cytochrome P-450. Under intracellular conditions, i.e. at about pH 6.9, cytochrome b_5 has to be assumed to play an important role sustaining optimal activities of the microsomal cytochrome P-450 dependent monoxygenase system.

Effects of spironolactone and phenobarbital on plasma fibrinogen in rats

S. Tóth
State Hospital, Parádfürdő (Hungary)

Spironolactone and phenobarbital (20 µM/100 g b.wt) given to rats for 3 consecutive days are capable of inducing an elevated fibrinogen level. At the same time, an enhanced incorporation of C^{14}-amino acids into fibrinogen has been observed. The effect of phenobarbital was more pronounced than that of spironolactone. These drugs, known as microsomal enzyme inducers, influence the synthesis of an enzymatically inactive product of the liver.

Lead acetate-induced endotoxin-hypersensitivity

L. Bertók
'Frédéric Joliot-Curie' National Research Institute for Radiobiology and Radiohygiene, Budapest (Hungary)

Selye, Tuchweber and Bertok (1966) demonstrated that a single, normally well-tolerated, i.v. injection of lead acetate increases the sensitivity of the rat to endotoxin of various gram-negative bacteria about 100,000 times above normal. The sensitizing effect of lead acetate for endotoxin is the highest when two agents are given simultaneously. The lead acetate-induced endotoxin hypersensitivity has been confirmed by several authors (Berczi et al. 1967, 1968, DeClercq and Merigan 1969, Truscott 1970, Filkins 1970, 1973, Trejo et al. 1971, 1972, Rippe and Berry 1972, Hoffmann et al. 1972, Selyberth et al. 1972, Holper et al. 1973, Cook et al. 1973, 1974, 1978, 1982, Cornell and Filkins 1974, Kisida et al. 1974, Bailey 1976, Orban et al. 1978, Kerkvliet and Baecher-Steppan 1982, Sakaguchi et al. 1982). It was also demonstrated that sulfhydryl compounds (e.g. cysteine) and endotoxin tolerance inhibit this endotoxin hypersensitivity provoking effect of lead acetate (Bertók 1968, Cook and DiLuzio 1973). It has been proved that lead causes a considerable reduction in the defensive capacity against bacterial infections (Hemphille et al. 1971). It was also found that the lead acetate induced endotoxin hypersensitivity is a good tool for the demonstration of enteroendotoxemic origin of various shock conditions, e.g. intestinal ischemia (superior mesenteric artery occlusion), tourniquet shock, 'intestinal syndrome' of the radiation disease (Filkins and Buchanan 1973, Bertók and Kocsar 1974, Kisida et al. 1974, 1976, Orban et al. 1978).

Lead acetate also increases the endotoxin-induced fetopathy and abortus (Csordas and Bertók 1981). Moreover, the lead acetate-induced endotoxin hypersensitivity has been used to demonstrate minute quantities of endotoxin in biological materials (Kocsar et al. 1969, Bertók and Kocsar 1974, Kisida et al. 1974). The use of this method was very important in the recognition of endotoxin absorption from the intestine (the pathogenesis of enteroendotoxemic shock), the role of bile acids played in it and the discovery of the so-called physicochemical defense of macroorganisms (Kocsar et al. 1969, Bailey 1976, Bertók 1977).

On the other hand, the increasing pollution of the environment caused by lead raised the possibility of interaction. Finally, on the basis of experimental evidence, one can surmise the alterations of the detergent effect of bile acids plays an important role in the mechanism of lead acetate-induced endotoxin hypersensitivity (Bertók 1977, 1983).

Functional consequences of the reticuloendothelial blockade induced by gadolinium chloride

G. Lázár and E. Husztik
Institute of Pathophysiology, Institute of Medical Biology, University Medical School, Szeged (Hungary)

In studies of the physiologic and pathophysiologic roles of the reticulo endothelial system, the depression or blockade of the granulopectic activity of this system has attracted considerable attention. 10 years ago at Dr Selye's institute, it was found (Lázár 1973) that rare earth metal salts, among them gadolinium chloride, depress reticuloendothelial activity and inhibit or completely abolish the effects of some reticuloendothelial stimulants. We recently studied the cellular and humoral bases as well as some of the functional consequences of this form of reticuloendothelial blockade.

Experiments with heterologous erythrocytes and endotoxin labeled with ^{51}Cr showed that gadolinium chloride-induced reticuloendothelial blockade is due to depressed phagocytic activity of the Kupffer cells. Our light and electron microscopic investigations indicate that the failure of Kupffer cells to incorporate carbon during reticuloendothelial blockade induced by this rare metal salt is due to defects in the surface attachment and in the engulfment phases of phagocytosis.

Several data suggest that macrophages have important functions as accessory and regulatory cells in the induction and expression of the immune response. In mice pretreated with gadolinium chloride, significant increase of hemolytic titer to sheep red blood cells and increased number of hemolytic plaque-forming cells in the spleen were observed. Our experiments suggest that the increased humoral immune response in gadolinium chloride-pretreated mice is the consequence of the redistribution of the particulate antigen on the effect of the Kupffer cell phagocytosis blockade.

Our results support the involvement of reticuloendothelial function in anaphylaxis. Gadolinium chloride injected before the challenge of anaphylaxis to mice sensitized to ovalbumin greatly reduced anaphylactic death and the symptoms of anaphylaxis.

Morphological studies of diesel particulate clearance from rat lungs

B. D. Garg
Biomedical Science Department, General Motors Research Laboratories, Warren (Michigan 48090, USA)

Diesel engines produce particulate emissions which can deposit in the alveolar regions of the lung after inhalation. Particulate matter in diesel engine exhaust consists of hydrocarbon-coated carbonaceous material in the respirable size range of 0.2 μm. This paper presents the results of some morphological studies on the clearance mechanisms of the rat lung. In one series of experiments, groups of rats were exposed to diesel exhaust at 250, 750, 1500 $\mu m/m^3$ for up to 45 weeks and to 6000 $\mu g/m^3$ for up to 9 weeks. Each group was sacrificed immediately postexposure and pulmonary structures were examined histologically. Diesel particulates were observed in pulmonary macrophages of all animals exposed to the diesel exhaust. The number of alveolar macrophages was increased with exposure in a manner that appeared to be dose dependent and could be identified in alveoli, terminal bronchiolar region and in the bronchi. In addition, diesel particulate-laden macrophages and some free diesel particulates were observed in the BALT (bronchus associated lymphoid tissue) and in the mediastinal lymph nodes. From these observations, we conclude that both the mucociliary clearance process and the lymphatic system contribute to the removal of diesel particulates from the lung. However, not all pulmonary macrophages were removed from the lung by these processes. Some uncleared particulate-laden macrophages in the lung formed macrophage aggregates (MA). By contrast, no MA were observed in the lungs of rats exposed to filtered room air. To characterize the MA accumulations, a preliminary morphometric study was performed. Rats were exposed to 6000 $\mu g/m^3$ for 2 weeks. One group of six rats was sacrificed immediately after exposure (0-week postexposure) and a second group of 6 rats was sacrificed 6 weeks after discontinuation of exposure (6-week postexposure). In the 0-week postexposure group, the total number of aggregates was 300,000 ± 18,000 with a mean diameter of 20.4 ± 0.5 μm and a maximum of 48.1 μm. The average volume of MA was 5,407 ± 380 μm^3 and the lung volume occupied by the aggregates was 0.02% of the total lung volume. In the 6-week postexposure group, the total number of aggregates was 270,000 ± 19,000 with a mean diameter of 30.5 ± 0.6 μm and a maximum of 129.0 μm. The average volume of a MA in this group was 32,855 ± 2720 m^3 and the lung volume occupied by the aggregates was 0.12% of the total lung volume. The diameter of MA was significantly higher in the 6-week postexposure group as compared to th 0-week postexposure group. The increase in diameter of macrophage aggregates after cessation of diesel exhaust exposure indicates a fusion of some of the unaggregated macrophages not removed by mucociliary or lymphatic clearance.

Magnetic stimulation of polymer accumulation in bacterial cultures

V. W. Adamkiewicz and D. R. Pilon
Departments of Microbiology and Immunology, University of Montreal, Montreal (Quebec, Canada H3C 3J7)

While investigating 'dental plaque', in vitro, we noticed in 1979 that more plaque accumulated on one side of glass slides. The magnetic nature of this preferential accumulation (P.A.) was established as follows. Pairs of microscope slides, stuck back-to-back with a drop of water, were incubated vertically in cultures of *Streptococcus mutans* (ATCC, 25175, 'C'), growing in BHI broth plus 10% sucrose, at 37 °C, 24 h, in the dark. Streptococcal exopolysaccharides, that adhered irreversibly, were determined separately on each slide of the pair, by electrophotocolorimetry. Polysaccharide accumulation is up to 100% greater on slides facing north rather than south. This P.A. is proportional to the angle of slide and geomagnetic meridian. Reversal (in solenoid coil) of magnetic field by 180° causes similar reversal of P.A. At 'zero field' north and south slides accumulate equivalent amounts. P.A. is a physiological reaction. It occurs in weak, static, geomagnetic-like fields. It becomes saturated in fields 1.7 g. P.A., is up to 100% greater on metallic *mutans*, is non motile, and contains no magnetosomes. P.A. appears limited to adhering polysaccharides, and to modulate adsorptions to vertical surfaces. (We thank the Dean, Pierre Bois, for support.)

Survival and long-term sequelae in very low birth weight infants

F. Lefebvre and A. Veilleux
Service de Néonatologie, Hôpital Ste-Justine, and University of Montreal, Montreal (Quebec, Canada H3T 1C5)

Neonatal charts were reviewed to assess the survival rate of very low birth weight (VLBW) infants (501–1000 g) during a 4-year period (1976–1979) in a large neonatal intensive care unit. VLBW infants born alive in this center (n = 121) and infants

referred to the unit (n = 120) had an overall survival rate of 19.1% (46/241). Survival below 751 g birth weight was infrequent (2.6%). Survivors were heavier (914 ± 77 vs 792 ± 137 g), had greater gestational age (29.0 ± 2.6 vs 26.3 ± 2.4 w), more intrauterine growth retardation (39.1 vs 20.3%), more cesarean births (37.2 vs 9.7%) and less asphyxia (23.8 vs 66.1%) than infants who died. Assisted ventilation with endotracheal intubation was seldom used in either group (15.2 vs 29.2%).

To determine their quality of life, the 22 boys and 24 girls who survived were enrolled in a follow up program. Follow up for at least 2 years was obtained for 91.3% (42/46) of the survivors (mean age: 49.5 ± 15.2 months). Readmissions occurred in 45% of the children mainly for surgery and respiratory problems. At a mean age of 4 years, growth retardation was present in 38.1% of the children, more often in males (66.7%) and in those who had intrauterine growth retardation (60.0%); microcephaly was present in 19.0%, mainly in males (33.3%). Only mild grade retinopathy of prematurity was detected in 7.1%. Neuropsychiatric handicaps were: profound sensorineural deafness (7.1%), cerebral palsy (9.5%), hydrocephaly (2.4%), epilepsy (4.8%) and severe psychiatric disorders (4.8%). Mean developmental quotient (DQ) was 95.0 ± 13.9 (range 61.9–124.5) for the 24 children tested. Three children had mental retardation and four had a borderline DQ (71–84). Two additional children had a language delay in a screening test. Two children with normal D.Q. had learning disabilities. Overall handicaps were severe in 16.7%, mild to moderate in 16.7%, and 66.6% of the children were considered normal. Survivors with sequelae had a younger gestational age, were more often males, less often growth retarded in utero, needed a longer period of parenteral nutrition and had more neonatal seizures than normal survivors. The actual more aggressive approach toward management of VLBW infants including long-term ventilator therapy needs continuing assessment of the quality of life of the survivors.

Role of tocopherol in carcinogenesis

P. Constantinides
Pathology Department, Louisiana State University Medical School, Shreveport (Louisiana 71130, USA)

When α-tocopherol (vitamin E), dissolved in soya oil, was given to 22 Balb/c mice once a week s.c. for 500 days, it caused the local development of extremely vigorously growing fibrosarcomata in 80% of the animals. The tumors produced in this manner grow from pin-head size to half to ⅔ the size of a whole mouse within 3 weeks when transplanted into isogeneic hosts. They are true neoplasms and have been serially transplanted for about 2½ years. They are now in their 34th transplantation cycle. Neither pure α-tocopherol alone nor soya oil alone produced any tumors when given s.c., once a week, for 500 days to groups of 22 Balb/c mice each. We conclude that two completely noncarcinogenic agents (tocopherol and soya oil) can develop a powerful carcinogenic effect when acting on tissues simultaneously. The theoretical interest of this conclusion is that until now non-carcinogens (such as e.g. croton oil) were known to be capable of 'promoting' the action of carcinogens, but not of initiating carcinogenesis themselves. This is the first time, to our knowledge, that noncarcinogens have been found capable of 'initiating' carcinogenesis under certain conditions.

Osmotically-induced oncolysis

R.H. Orvoine
Department of Microbiology and Immunology, University of Montreal, Montreal, (Quebec, Canada H3C 3J7)

The basic mechanisms involved in the acute conditioned necrosis of the skin (ACN) as described by Selye et al. (1966) may be applied to the local destruction of solid tumors. A strongly hypertonic environment is lethal to cells if return to isotonicity is prevented by local ischemia obtained by vasoconstriction soon followed by thrombosis. Serotonin (5-HT) plays a major role by constricting arterioles, increasing the permeability of venules and activating the coagulation cascade. A high concentration of glucose further contributes to the development of thrombosis by increasing the viscosity of plasma, altering the vascular endothelium and the membrane of blood cells. Exposed to a stable hypertonic and anoxic milieu, tumoral cells and their surrounding connective tissue rapidly suffer irreversible damage. 20–30 min after injection of a mixture of 5-HT and hypertonic glucose into and around a 1.5x1.5 cm s.c. tumor, cyanosis of the skin covering and immediately surrounding the growth is evident and is soon followed by frank necrosis. In a large number of cases, tumors disappear within a few days after a single treatment. Electron microscopy confirms the rapidity of the necrotic process. Systemic side effects of the therapy consist mainly of cellular dehydration with expansion of the extracellular space.

Records and research

E.C. Hay
Scientific Information Center, Ciba-Geigy Pharmaceutical Division, Summit (New Jersey 07901, USA)

In his research, Dr Selye used and created records in large numbers over a period extending from 1929 to 1982. The record keeping of his students helped him to create the manuscripts that he published. The manner in which he organized research; the method by which he indexed the findings of his own laboratory and those of others has influenced the lives of his students, the language of biomedical documentation and the treatment of patients. The drama of his popular communications changed the beliefs and behaviors of countless people throughout the world. Records to him were a means to an end. He thrived on controversy.

During these years, the 'careful observation' aspects of research in 'developed countries' has become increasingly expensive, more and more concentrated in large governmental, industrial and academic laboratories; and less and less the endeavor of an individual in intimate contact with another human, an animal, a plant, a mountain, a river, a tundra, a snowflake, a butterfly, a contaminated Petri dish. Experiments are performed all too often to make records required by a government agency. Such research resembles a production line with the report the product. Records for records sake smother innovation.

I urge that we honor Dr Selye as individuals and as groups by encouraging persons so compelled to explore the unusual or the controversial that they sacrifice to investigate it. Dr Frederick Banting was no great scholar but in the course of his medical practice he acquired an overwhelming idea. He gave up security to pursue it. The initial observations that led to the concepts of the General Adaptation Syndrome and the physiology and pathology of stress were not carried out by a secure scientist in a well equipped environment. They originated in a prepared mind dedicated to the intense and intimate manipulation and observation of experimental materials. Records were used not to justify but to authenticate, communicate and convince.

The 'G-G' test

P. Rohan
INRS-Santé (UQ) and McGill University, Montreal (Quebec, Canada)

Various tests and biological models have been developed in order to demonstrate metabolic, toxic, mutagenic, teratogenic, cancerogenic and other environmental effects on living structures. The proposed gerontogenic or 'G-G' test represents a complementary biological model assessing the quality of the

environment, as reflected upon the phenomenon of accelerated experimental 'aging'. Its concept is based upon the very old observation that during the process of aging, a considerable shift of minerals exists from 'hard' structures towards 'soft' tissues, resulting in an increased vulnerability of brittle bones and teeth as well as in growing pathological incrustations of arteries, periarticular tissues and the crystalline lens of the eye by calcium salts (after previous degenerative changes), in some relation with the phenomenon of 'natural' aging. The 'Progeria-like syndrome' observed by Hans Selye in rats and studied in detail by Tuchweber, Selye and his coworkers, may gain importance, in view of future ecologic studies. Some theoretical and practical aspects of the proposed environmental 'G-G' test are initiated.

Acknowledgments
The Organizing Committee of the Symposium Hans Selye (Drs A. Robert, S. Szabo and Y. Taché) would like to acknowledge the financial and organizational support received from the Hans Selye Foundation. We are especially indebted to its president Mr M. Cantin and to Mme Louise Drevet-Selye. Université de Montréal also provided important support and the organizational expertise of Dr G. Plaa, vice-president was invaluable for the planning and execution of the symposium. We also thank for the hospitality and gratifying help provided by Dr Y. Gauthier, dean of the Faculté de Médecine.
We express our gratitude to companies and institutions that provided crucial financial support: Bank of Montreal, Fonds de la Recherche en Santé du Québec, Glenbrook Laboratories (Division of Sterling Drug, Inc.), Hoffmann-La Roche, Medical Research Council of Canada, C.V. Mosby Publishing Company, G.D. Searle and Company, Serono Laboratories, Smith Kline and French Laboratories, Van Nostrand Reinhold Company (Scientific and Academic Editions) and Williams & Wilkins Publishers.
The following persons also earned our sincere appreciation with their help in the organization of the symposium and processing of the material for publication: Yolande Côté, Ginette Larue, Irène Mécs and Ovid Da Silva.

'Waldsterben': Our Dying Forests – Part II

Implications of the chemical soil conditions for forest decline

E. Matzner and B. Ulrich

Institut für Bodenkunde und Waldernährung, Universität Göttingen, Büsgenweg 2, D-3400 Göttingen (Federal Republic of Germany)

Key words. Forest decay; soil acidification; predisposing stresses; incitant stresses; contributing stresses.

Introduction

The forest decline which has become evident in many parts of Central Europe is of a complex nature[42]. It cannot be attributed to a single stress factor, but must be related to the interaction of a number of stress factors of abiotic and/or biotic origin.
In order to elucidate the relationship between different stress factors and forest decline, Manion[19] has developed a concept which describes forest decline. He also mentions one characteristic phenomenon of forest decline, which is the lack of agreement between various researchers about the 'cause' of the decline and the importance of the specific stress factors involved. Each investigation attempts to find the 'cause'.
To explain processes of complex origin such as forest decline, Manion distinguishes between three groups of stresses; predisposing, inciting and contributing stresses. Predisposing stresses are long-term factors which are relatively static, or unchanging, such as climate, soil type, the genetic potential, the age of the tree and the long-term effects of air pollutants. The last point covers, in our opinion, the chronic effects of low concentrations of gaseous pollutants as well as those of long-lasting proton input into forest soils from the atmosphere or from internal production. This aspect will be discussed later. Predisposing factors put a permanent stress on the plant and weaken it in such a way that other factors can become effective.
The second group of stresses is called 'incitant'. These stresses are short in duration and may be abiotic or biotic in nature. Examples of incitants are insect defoliators, frost, drought, salt spray, and the short-term effects of high concentrations of air pollutants. They generally produce a drastic injury. The plant attempts to recover but has difficulties because of the presence of the predisposing stress. We think that the soil acidification pushes arising within the soil from favorable climatic conditions have to be added here. This factor will also be discussed later.
The third group of stresses, called 'contributing', finally begin to appear. Bark beetles, cancer fungi, root and sap rot fungi, viruses and mycoplasms produce noticeable symptoms and signs on the weakened host. These organisms are persistent and are often blamed for the condition of the hosts.
The concept given by Manion provides a framework to cover all hypotheses on forest dieback produced so far.

Soil acidification as a predisposing stress

The acidification of a soil may act as a predisposing stress for the plant in a general way by causing a reduction in nutrient availability and/or by production of toxic ions in the soil solution leading to decreased root growth and ion uptake. For some elements (e.g. Ca and Mg) shortage and toxicity in the soil are interrelated and could be enhanced by each other[39]. Predisposing stress has been